228° (from EtOH), undepressed on admixture with an authentic sample of (IX). Yield, 1.0 g. Evaporation of the mother liquor and recrystallization of the residue from EtOH gave colorless plates, m.p. 156~158°, undepressed on admixture with an authentic sample of (XIII). Yield, 0.9 g.

The authors' thanks are due to the members of the Microanalytical Center of this Institute for microanalyses. They are also grateful to Mr. K. Machida and Miss I. Uchida for the measurement of infrared absorption spectra.

Summary

1-Substituted 6-chloro-1*H*-imidazo[*b*]pyridine was prepared. The Mannich reaction of 6-bromoimidazo[*b*]pyridine and the behavior of its product in the alkylation reaction were described.

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95. Morizo Ishidate, Yoshio Sakurai, and Yutaka Kuwada : Studies on Carcinostatic Substances. XXIX.*1 1,1-Bis(2-chloroethyl)-hydrazine and its Derivatives as Tumor-inhibiting Agent.

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

At present, in the field of study on preparing the anti-tumor derivatives of 2-chloroethylamine, it seems to be a new tendency to seek out derivatives with latent activity in order to improve their efficacy, i.e. to elevate their selectivity of action on the tumor tissues. In most works, however, they were confined to derivatives which might release the secondary amine, viz. bis(2-chloroethyl)amine, *in vivo* as an active component.

Studies have long been made to prepare compounds with latent activity, viz. masked derivatives, which could be activated by reduction *in vivo*, yielding the corresponding tertiary bis(2-chloroethyl)amine. Among them, N-methyl-bis(2-chloroethyl)amine N-oxide and N,N-bis(2-chloroethyl)isoxazolidinium halide[1] were proved so far to be the most promising, at least in animal experiments.

In this paper, an experiment to prepare the substituted derivatives of 1,1-bis(2-chloroethyl)hydrazine is described. A brief report[2] was already published in 1959 by the authors on the preparation of 1,1-bis(2-chloroethyl)hydrazine itself, because it was found at that time that Preussman[3] had independently published his work on the same compound.

This compound was found to have a strong biological activity, but it was particularly noticed that its chemical and biological activities diminished or were totally lost by substitution of its primary amino group with any other substituent. From this observation, it could be anticipated that an effective masked compound might be found among its

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*1 Part XXVIII. H. Imamura: This Bulletin, 8, 449(1960).

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1) T. Yoshida, M. Ishidate, Y. Sakurai, *et al.*: Proc. Japan. Cancer Assoc., 17th General Meeting, November, 1958.
2) M. Ishidate, Y. Sakurai, Y. Kuwada : This Bulletin, **7**, 391(1959).
3) R. Preussmann, *et al.* : Angew. Chem., **70**, 743(1958).

derivatives, if it were possible to arrange their chemical structure so as to be activated *in vivo* by releasing the additive component in the molecule.

This study deals with the preparation and investigation of the chemical and biological activities of 1,1-bis(2-chloroethyl)hydrazine and its hydrazone- or hydrazide-type derivatives.

The process of synthesis was as follows:

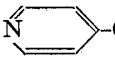
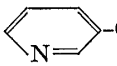
$$R\text{-}NHNH_2 \xrightarrow{CH_2\text{—}CH_2 \backslash O /} R\text{-}NHN(CH_2CH_2OH)_2 \xrightarrow{SOCl_2} R\text{-}NHN(CH_2CH_2Cl)_2$$

$$\longrightarrow NH_2N(CH_2CH_2Cl)_2 \qquad R = C_6H_5CO\text{-} \text{ or } CH_3CO\text{-}$$

Reduction of N-nitroso-bis(2-chloroethyl)amine with hydrogen over catalyst or with zinc and acetic acid was also tried, but the results were always complicated and no simple product could be isolated even after repeated trials under various conditions.

The hydrazides were obtained by mixing and heating with the corresponding acid chloride without addition of alkali or any other base, and the hydrazone by the usual procedure.

Table I.

No.	Compound			*in vitro* MEC[c]		*in vivo* (mg./kg.)			
				CE	m*M*/L.	CE	LD_{50}	MTD	MED
(I)	$NH_2N(CH_2CH_2OH)_2$		b.p_{12} 155~157°	−					
(II)	$NH_2N(CH_2CH_2Cl)_2HCl$		m.p. 132~133°	+	5×10^{-3}	+	7.5	5	0.5
	$R\text{-}NH\text{-}N(CH_2CH_2Cl)_2$								
	R		m.p.						
(III)	C_6H_5CO-		102~103	+	2.5×10^{-2}	+	175	100	50
(IV)	[a]		144~145	−					
(V)	CH_3CO-		76~77	+	2.5×10^{-2}	+	175	100	50
(VI)	3,5-$(NO_2)_2C_6H_3CO$-		153~154 (decomp.)	−					
(VII)	$ClCH_2CO$-		61~62	+	1×10^{-2}	−	30	10	
(VIII)	Cl_2CHCO-		78~79	+	2.5×10^{-2}	+	75	50	50
(IX)	$Cl_3C\text{-}CO$-		90~91	+	2.5×10^{-3}	−	7.5	5	
(X)	OHC-		77~78	−		+	7.5	5	5
(XI)	C_2H_5OCO-		77~78	+	5×10^{-2}	+	75	50	10
(XII)	 -CO-	HCl	Syrup	+	2.5×10^{-2}	+	375	250	50
		Picrate	169~170 (decomp.)	+	1×10^{-1}				
(XIII)	 -CO-	HCl	Syrup	+	2.5×10^{-2}	+	175	100	10
		Picrate	154~155	+	1×10^{-1}				
(XIV)	 CH_2CO-	Picrate	80~81	−					
(XV)	NH_2CO-		119~120	−		+	375	250	50
(XVI)	C_6H_5NHCO-		94~95	−					
(XVII)	C_6N_5HHCS-		125~126	−					
(XVIII)	$(ClCH_2CH_2)_2NNHCS$-		144~145	+	2.5×10^{-2}	+	175	100	5
	$R{=}N\text{-}N(CH_2CH_2Cl)_2$								
	R								
(XIX)	$(CH_3)_2C{=}$	HCl	150~151	+	2.5×10^{-3}	+	75	50	5
(XX)	$C_6H_5CH{=}$		Yellow oil	+	1×10^{-1}	+	750	500	100
(XXI)	*o*-$HO\text{-}C_6H_5CH{=}$		〃	+	1×10^{-1}				
(XXII)	$CH_3C(C_6H_5){=}$		〃	+	2.5×10^{-2}	+	375	250	100
(XXIII)	*p*-$(CH_3)_2NC_6H_4CH{=}$	HCl		+	2.5×10^{-2}	+	375	250	10
		Picrate	165~166 (decomp.)	+	5×10^{-2}	+			
(XXIV)	$CH_3C(COOH){=}$				5×10^{-2}	+	7.5	5	1
		Salt[b]	147~148 (decomp.)	+	5×10^{-3}				

a) 2-Phenyl-4-(2-chloroethyl)-5,6-dihydro-1,3,4-oxadiazine hydrochloride

b) S-Benzylthiuronium salt *c*) Minimum effective concentration (MEC) in m*M*.

Table II. Cl^- Liberation and Thiosulfate Consumption at 37°

No.		10 (min.)	30	1 (hr.)	2	5	24	48	72	96	120
(II)	Cl	0.01	0.25	0.28	0.38	1.25	1.63				
	S_2O_3	—	—	—	—	—	—				
(III)	Cl	0	0	0	0	0.14	0.47	0.72	0.92	1.07	1.20
	S_2O_3	0	0	—	0	—	0.16	0.32	0.44	0.56	0.67
(V)	Cl	0	0	0	0.04	0.08	0.37				
	S_2O_3	0	0	—	0	—	0.20				
(VII)	Cl	0	0	0	0	0.13	0.39				
	S_2O_3	0.32	0.51	—	0.80	—	0.80				
(IX)	Cl	1.85	1.85	2.04	2.34	2.34	2.63				
	S_2O_3	1.88	1.88	—	1.88	—	1.58				
(X)	Cl	0	0	0	0	0.03	0.20				
	S_2O_3	0	0	—	0	—	0.12				
(XI)	Cl	0	0	0	0	0.02	0.15				
	S_2O_3	0	0	—	0	—	0.20				
(XII)	Cl	0	0	0	0	0	0				
	S_2O_3	0	0	—	0	—	0.02				
(XIII)	Cl	0	0	0	0	0	0				
	S_2O_3	0	0	—	0	—	0.11				
(XV)	Cl	0	0	0	0	0	0.03	0.06	0.08	0.12	0.12
	S_2O_3	0	0	—	0.01	—	0.06	0.13	0.17	0.25	0.30
(XVIII)	Cl	0	0	0	0	0	0				
	S_2O_3										
(XIX)	Cl	0.01	0.01	0.08	0.35	1.35	1.79				
	S_2O_3	—	—	—	—	—	—				
(XXII)	Cl	0	0	0.02	0.02	0.05	0.18				
	S_2O_3	0	0	—	0.01	—	0.17				
(XXIII)	Cl	0	0	0	0	0	0				
	S_2O_3	0	0	—	0	—	0.38				

Biological activity of the compounds on the Yoshida sarcoma rats is summarized in Tables I and II, together with the result on titration of liberated Cl^- and thiosulfate uptake *in vitro*.

From these results, it was known that most of the compounds exhibited an extremely slow Cl^- liberation and thiosulfate uptake, though thiosulfate determination with iodine solution was not applicable in the case of (II) and (XIX), since they themselves consumed iodine. Rapid and excess Cl^- liberation and thiosulfate uptake of (IX) was an exception. It seemed to be due to hydrolysis and alkylation of the chlorine atoms in its acyl group. In spite of their chemical inertness, most of the compounds were found to be active against the tumor and clearly showed their masked character.

They were investigated with the *in vitro*-cultured Yoshida sarcoma cells by the routine procedure,[4] the results of which are also indicated by the minimum effective concentration (MEC) in Table I.

Comparing the effectiveness determined *in vitro* and *in vivo*, it is found that some hydrazide-type derivatives were ineffective or less effective than the hydrazone-type, against the *in vitro* tumor although they appeared equally effective against the *in vivo* tumor. This might suggest that the latter derivatives are apt to release the active component by the action of tumor cells themselves more easily than the former, but in the animal body both types of derivatives seemed to be subject to almost equal activation. In fact, (XIII) was ineffective against the *in vitro* tumor under contact conditions (37°, 120 min.) up to the concentration of 10^{-1} m*M* as shown in Table III, contrary to (XIX) which gave MEC of 10^{-2} m*M* even on milder contact (37°, 30 min.). Results of such activation

4) M. Ishidate, *et al.*: This Bulletin, **7**, 873(1959).

Table III.

Compound	Contact time at 37° (min.)	MEC			
		$(5\times10^4)^{a)}$	(5×10^5)	(5×10^6)	(5×10^7)
(III)	30	—	—	—	—
(V)	30	(1×10^5)	(1×10^6)	(1×10^7)	(1×10^8)
		—	—	—	—
(XIII)	120	(1×10^5)	(1×10^6)	(1×10^7)	(1×10^8)
		—	—	—	—
(XV)	120	(1×10^5)	(1×10^6)	(1×10^7)	(1×10^8)
		—	—	—	—
(XIX)	30	(1×10^5)	(1×10^6)	(1×10^7)	(1×10^8)
		10^{-2}	10^{-2}	10^{-2}	10^{-2}

a) (Cell no./cc.) in the medium.
— : No effect at concentration from 10^{-1} to 10^{-4} m*M*.

experiments, employing the technique[5] reported by the authors, are demonstrated in Table III.

It might be concluded therefore that the unmasking mechanism of the hydrazide seemed to depend less on the biological action of the tumor cell than on that of the organs or tissues of the tumor-bearing animals, because the effect was not influenced by the cell population (cell no./cc.) as seen in Table III. For example, (X) and (XV) were found to be effective only in *in vivo* experiment, while (VII) and (IX) exhibited their effect only in the *in vitro* experiment. For this, the explanation might be, that the activation of these compounds was chiefly due to chemical hydrolysis into their components, viz. acid and hydrazine, but the reaction velocity was too slow to accumulate the active components in the animal body before they were detoxicated or excreted. Indeed it could be said that the time during which the tumor cells were kept in contact with the chemical agent was generally far longer in the *in vitro* test than in the *in vivo* experiment.

Percentage survival diagrams of (II) and (XIII) were drawn with 12 Yoshida sarcoma rats each, and are presented in Figs. 1 and 2. Both results indicated, however, that these two compounds were no match for N-methyl-bis(2-chloroethyl)amine N-oxide as a tumor-inhibiting agent.

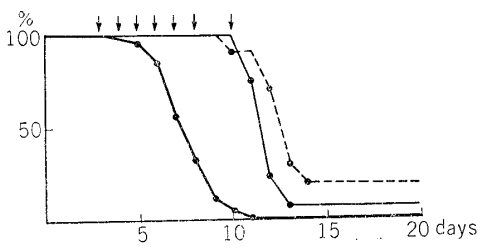


Fig. 1. Percentage Survival Diagram with (II)
— Control
—— 1 mg./kg. i.p.×7
------ 2.5 mg./kg. i.p.×7

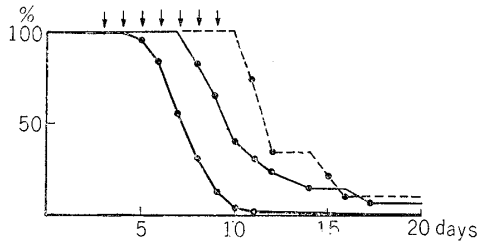


Fig. 2. Percentage Survival Diagram with (XIII)
— Control
—— 20 mg./kg. i.p.×7
------ 50 mg./kg. i.p.×7

In order to compare the properties of the hydrazides with the amides of the same acids, a few amides of bis(2-chloroethyl)amine were investigated as to their Cl^- liberation *in vitro* and biological activity. As seen in Table IV, all amides were found completely inactive on the tumor either *in vitro* or *in vivo*. From this experience, it was thought that the masking of hydrazide-type was rather more promising than the amide-type.

Preparation of α-amino acid hydrazides derived from (II) is now proceeding.

5) H. Imamura : This Bulletin, 8, 449(1960).

Table IV.

No.	R-N(CH_2CH_2Cl)$_2$ R	b.p. (°C/mm.Hg)	*in vitro* MEC CE	*in vitro* MEC m*M*/L.	*in vivo* (mg./kg.) CE	LD$_{50}$	MTD	MED
(XXV)	CH_3CO-	150/12	−					
(XXVI)	$ClCH_2CO$-	167~169/8	−					
(XXVII)	C_2H_5OCO-	117~120/7	−		−	375	250	
(XXVIII)	NO-	Yellow oil	+	2.5×10^{-2}	+	175	100	10
(XXIX)	*p*-$CH_3C_6H_4SO_2$-	m.p. 48~49					>1000	

No.	Cl^- liberation at 37° 10 (min.)	30	1 (hr.)	2	5	24
(XXV)	0.08	0.27	0.44	0.63	1.29	1.66
(XXVI)	1.51	1.62	1.64	1.69	1.69	1.70
(XXVIII)	0	0.01	0.02	0.04	0.06	0.28

Experimental

1,1-Bis(2-hydroxyethyl)hydrazine (I)—Ethylene oxide prepared from ethylene chlorohydrin (91 g.) by the usual method was passed through a mixture of hydrazine hydrate (18 g.) and H_2O (20 cc.) under violent stirring at 10~13° and then kept standing overnight in an ice box with a rubber stopper. After evaporation, the residue was distilled *in vacuo*. b.p$_{12}$ 155~157°.[6]

1-Benzoyl-2,2-bis(2-hydroxyethyl)hydrazine—Benzoylhydrazine (40 g.) was added into *N* AcOH (360 cc.). Ethylene oxide prepared from ethylene chlorohydrin (141 g.) was passed into a mixture at 30° and, after it was kept stoppered in an ice box for 36 hr., H_2O was removed by distillation. The crystalline residue was recrystallized from AcOEt to white prisms, m.p. 87~88°. Yield, 45 g. *Anal.* Calcd. for $C_{11}H_{16}O_3N_2$: C, 58.91; H, 7.19; N, 12.49. Found: C, 58.90; H, 7.11; N, 12.20.

1-Benzoyl-2,2-bis(2-chloroethyl)hydrazine (III)—A solution of 1-benzoyl-2,2-bis(2-hydoxyethyl)-hydrazine (19 g.) in $CHCl_3$ (60 cc.) was dropped into a mixture of $SOCl_2$ (35 cc.) and $CHCl_3$ (20 cc.) below 10° and the mixture was kept standing at room temperature for 36 hr. with occasional shaking. After evaporation of $SOCl_2$ together with the solvent and washing the residue with Et_2O, it was separated by addition of EtOH into insoluble (A) and soluble (B) portions. (A) was removed by filtration and the filtrate was diluted with H_2O after treating with activated carbon. A brown crystalline mass separated, which was extracted with hexane. After removal of hexane, colorless crystals were obtained and recrystallized from petr. ether to white needles, m.p. 102~103°. Yield, 8.8 g. It was insoluble in H_2O and 5% HCl but gradually soluble in 10% HCl and did not reduce the Fehling solution. *Anal.* Calcd. for $C_{11}H_{14}ON_2Cl_2$: C, 50.59; H, 5.40; N, 10.73. Found: C, 50.42; H, 5.04; N, 10.73.

(III) was also obtained when a mixture of (II) (1 g.), BzCl (1 cc.), and dry benzene (5 cc.) was refluxed on a boiling water bath for 3 hr. It was treated as for (V). The mixed m.p. of the two specimens prepared by the two different processes showed no depression.

(A) weighed about 7 g. which was washed with Et_2O and recrystallized from Me_2CO to white plates, m.p. 144~145°. Yield, 3.3 g. It gave analytical values equal to those of (III), but was quite different in solubility in H_2O. It was easily soluble in H_2O and from the solution, AgCl precipitated at once when $AgNO_3$ solution was added. From its analytical data, it was identified with 2-phenyl-4-(2-chloroethyl)-5,6-dihydro-1,3,4-oxadiazine hydrochloride (IV). *Anal.* Calcd. for $C_{11}H_{14}ON_2Cl_2$: C, 50.59; H, 5.40; N, 10.73; Cl, 27.16; Cl^-, 13.58. Found: C, 50.62; H, 5.23; N, 10.52; Cl, 27.59; Cl^-, 13.51.

1,1-Bis(2-chloroethyl)hydrazine Hydrochloride (II)—(III) (10 g.) was heated with conc. HCl (40 cc.) for 10~17 hr. on a boiling water bath. The reaction mixture was diluted with 200 cc. of H_2O and extracted with Et_2O to remove both unchanged starting material and BzOH formed. After treating with activated carbon, aqueous layer was evaporated to dryness. The residue was recrystallized from EtOH-Et_2O to white scales, m.p. 132~133°. Yield, 3.6 g. *Anal.* Calcd. for $C_4H_{11}N_2Cl_3$: C, 24.83; H, 5.73; N, 14.48. Found: C, 24.86; H, 5.61; N, 14.38.

Its free base solidified as a colorless crystalline mass melting at about 100~110°, which could not be purified due to its instability. Its solution in anhydrous organic solvent seemed rather stable. The picrate, styphnate, or flavianate was not obtained in crystalline state.

From the Et_2O extract of the reaction mixture, 2.4 g. of unchanged starting material and 2.6 g.

6) b.p$_{25}$ 188~190° according to L. Knorr and H. W. Brownsdon (Ber., **35**, 4474(1902)).

of BzOH were recovered.

1-Acetyl-2,2-bis(2-chloroethyl)hydrazine (V)—A mixture of (II) (1 g.), AcCl (2 cc.), and dehyd. benzene (5 cc.) was refluxed for 3 hr. Crystals of (II) disappeared after a while with evolution of HCl. The reaction mixture was washed with 5% $NaHCO_3$ solution and H_2O, dried over anhyd. Na_2SO_4, and evaporated to dryness. The residue was recrystallized from hexane to white prisms, m.p. 76~77°. Yield, 0.7 g. *Anal.* Calcd. for $C_6H_{12}ON_2Cl_2$: C, 36.20; H, 6.08; N, 14.07. Found: C, 36.13; H, 5.82; N, 14.08.

1-(3,5-Dinitrobenzoyl)-2,2-bis(2-chloroethyl)hydrazine (VI)—Prepared similarly as in the case of (V), by heating a mixture of (II) (0.5 g.), 3,5-dinitrobenzoyl chloride (0.6 g.), and dehyd. benzene (10 cc.). Yield, 1 g. Pale yellow needles, m.p. 153~154° (decomp.), from EtOH. *Anal.* Calcd. for $C_{11}H_{12}O_5N_4Cl_2$: C, 37.62; H, 3.44; N, 15.96. Found: C, 37.56; H, 3.50; N, 16.04.

1-Chloroacetyl-2,2-bis(2-chloroethyl)hydrazine (VII)—Prepared by heating a mixture of (II) (1 g.), chloroacetyl chloride (1 g.), and dehyd. benzene (10 cc.). White needles, m.p. 65~66°, from hexane. Yield, 1.5 g. *Anal.* Calcd. for $C_6H_{11}ON_2Cl_3$: C, 30.86; H, 4.78; N, 12.00. Found: C, 30.78; H, 4.65; N, 12.17.

1-Dichloroacetyl-2,2-bis(2-chloroethyl)hydrazine (VIII)—Prepared by heating a mixture of (II) (0.75 g.), dichloroacetyl chloride (1 g.), and dehyd. benzene (10 cc.). White needles, m.p. 78~79°, from 50% EtOH. Yield, 0.7 g. *Anal.* Calcd. for $C_6H_{10}ON_2Cl_4$: C, 26.89; H, 3.76; N, 10.46. Found: C, 26.77; H, 3.77; N, 10.27.

1-Trichloroacetyl-2,2-bis(2-chloroethyl)hydrazine (IX)—Prepared by heating a mixture of (II) (0.75 g.), trichloroacetyl chloride (0.71 g.), and dehyd. benzene (20 cc.). White needles, m.p. 90~91°, from petr. ether. Yield, 0.9 g. *Anal.* Calcd. for $C_6H_9ON_2Cl_5$: C, 23.83; H, 3.00; N, 9.26. Found: C, 24.00; H, 3.13; N, 9.38.

1-Formyl-2,2-bis(2-chloroethyl)hydrazine (X)—A dry Et_2O solution of the free base prepared from (II) (0.7 g.) was added with formic acid (1 cc.). After evaporation of the solvent, it was warmed at 60° for 2 hr. on a water bath and then dried *in vacuo*. The residue was washed once with H_2O and recrystallized from 50% EtOH, m.p. 77~78°. Yield, 0.35 g. *Anal.* Calcd. for $C_5H_{10}ON_2Cl_2$: C, 32.45; H, 5.45; N, 15.14. Found: C, 32.42; H, 5.04; N, 14.95.

1-Ethoxycarbonyl-2,2-bis(2-chloroethyl)hydrazine (XI)—Prepared by heating a mixture of (II) (0.5 g.) and ethyl chloroformate (9.6 cc.) for 3 hr. on a boiling water bath. After evaporation to dryness *in vacuo*, the residue was extracted with Et_2O. White needles, m.p. 77~78°, from hexane. Yield, 0.5 g. *Anal.* Calcd. for $C_7H_{14}O_2N_2Cl_2$: C, 36.70; H, 6.16; N, 12.23. Found: C, 36.57; H, 5.93; N, 12.08.

1-Isonicotinoyl-2,2-bis(2-chloroethyl)hydrazine Hydrochloride (XII)—A mixture of (II) (1 g.), isonicotinoyl chloride hydrochloride (0.92 g.), and dehyd. benzene (10 cc.) was heated on a water bath for 2 hr. After removal of benzene *in vacuo*, the reaction product was dissolved in H_2O to which was added a solution of picric acid.

Picrate: Yellow needles, m.p. 169~170°, from EtOH. Yield, 1 g. *Anal.* Calcd. for $C_{16}H_{16}O_8N_6Cl_2$: C, 39.12; H, 3.28; N, 17.11. Found: C, 39.14; H, 3.21; N, 17.10.

The hydrochloride was used for animal experiment although it was not obtained in the analytically pure state.

1-Nicotinoyl-2,2-bis(2-chloroethyl)hydrazine Hydrochloride (XIII)—Prepared from (II) (0.5 g.) and nicotinoyl chloride hydrochloride (0.46 g.) by procedure similar to the preceding case. The reaction product was purified as a picrate of yellow needles, m.p. 154~155°, from EtOH. Yield, 1.2 g. *Anal.* Calcd. for $C_{16}H_{16}O_8N_6Cl_2$: C, 39.12; H, 3.28; N, 17.11. Found: C, 39.30; H, 3.26; N, 17.48.

The hydrochloride was not obtained in the analytically pure state.

1-{[2,2-Bis(2-hydroxyethyl)hydrazino]carbonylmethyl}pyridinium Chloride—Ethylene oxide prepared from ethylene chlorohydrin (16 g.) was passed into a solution of 1-(hydrazinocarbonylmethyl)-pyridinium chloride (5.3 g.) in H_2O (20 cc.) at 3~7° and the reaction mixture was kept standing overnight in an ice box with a well-fixed stopper. Water was removed and the residue was recrystallized from EtOH to hygroscopic, orange-brown plates, m.p. 164~165° (decomp.). Yield, 3.5 g. *Anal.* Calcd. for $C_{11}H_{18}O_3N_3Cl$: N, 15.24. Found: N, 14.86.

1-{[2,2-Bis(2-chloroethyl)hydrazino]carbonylmethyl}pyridinium Chloride (XIV)—The above hydrazide (2.2 g.) was added in portions into $SOCl_2$ (5 cc.) and the mixture was kept overnight at room temperature. After removal of the excess $SOCl_2$, the residue was dissolved in ice water, from which the picrate was obtained by usual method as yellow plates, m.p. 80~81°, from EtOH. *Anal.* Calcd. for $C_{11}H_{18}O_8N_6Cl_2$: C, 40.41; H, 3.59; N, 16.63. Found: C, 40.36; H, 3.57; N, 16.56.

1-Carbamoyl-2,2-bis(2-chloroethyl)hydrazine (XV)—To a solution of (II) (0.5 g.) in H_2O (2 cc.), 20% KCNO solution (1 cc.) was added and the mixture was warmed for a few min. on a water bath. White crystals separated as soon as it was cooled. White plates, m.p. 119~120°, from H_2O. Yield, 0.3 g. *Anal.* Calcd. for $C_5H_{11}ON_3Cl_2$: C, 30.02; H, 5.54; N, 21.00. Found: C, 30.16; H, 5.09; N, 20.71.

1-Phenylcarbamoyl-2,2-bis(2-chloroethyl)hydrazine (XVI)—To a dry Et_2O solution of the free base prepared from (II) (0.75 g.), phenyl isocyanate (0.45 g.), was added and the mixture was kept over-

night at room temperature. After removing the precipitated crystals, the solution was concentrated *in vacuo* and the residue was extracted with EtOH. The extract was diluted with H_2O. White prisms, m.p. 94~95°, from 50% EtOH. Yield, 1 g. *Anal.* Calcd. for $C_{11}H_{15}ON_3Cl_2$: C, 47.84; H, 5.47; N, 15.22. Found: C, 48.12; H, 5.18; N, 15.20.

1-Phenylthiocarbamoyl-2,2-bis(2-chloroethyl)hydrazine (XVII)—Prepared by a procedure similar to the preceding example by using phenyl isothiocyanate instead of phenyl isocyanate. White needles, m.p. 125~126°, from EtOH. Yield, 1 g. (from 0.75 g. of (II)). *Anal.* Calcd. for $C_{11}H_{15}N_3Cl_2S$: C, 45.21; H, 5.17; N, 14.38. Found: C, 45.11; H, 5.11; N, 14.19.

1,1,5,5-Tetrakis(2-chloroethyl)thiocarbohydrazide (XVIII)—A mixture of the free base, prepared from (II) (1 g.), and CS_2 (1 cc.) was kept at room temperature for 24 hr. The crystals that separated were collected and recrystallized from EtOH to white scales, m.p. 144~145° (decomp.). Yield, 0.5 g. Detection of S by Lassaigne was positive. It was insoluble in H_2O and 10% HCl. Its solution in 10% NaOH did not exhibit any color reaction with Na nitroprusside. *Anal.* Calcd. for $C_9H_{18}N_4Cl_4S$: C, 30.35; H, 5.09; N, 15.74. Found: C, 30.40; H, 4.88; N, 14.13.

1-Isopropylidene-2,2-bis(2-chloroethyl)hydrazine Hydrochloride (XIX)—A mixture of (II) (0.3 g.) and Me_2CO (3 cc.) was refluxed for 30 min. and then cooled. After removal of Me_2CO, the residue solidified. White plates, m.p. 150~151°, from Me_2CO. Yield, 0.2 g. *Anal.* Calcd. for $C_7H_{15}N_2Cl_3$: C, 36.00; H, 6.47; N, 12.00. Found: C, 35.77; H, 6.23; N, 12.07.

It was soluble in H_2O and reduced the Fehling solution. The aqueous solution precipitates AgCl at once on the addition of $AgNO_3$ solution.

1-Benzylidene-2,2-bis(2-chloroethyl)hydrazine (XX)—A dil. EtOH solution of (II) (1 g.), BzH (0.5 cc.), and AcOK (0.51 g.) was heated on water bath for 30 min. and the separated oil was extracted with Et_2O. Removal of Et_2O gave a yellow oil (0.9 g.). Both the Beilstein test and Dragendorff's color reaction of this sample appeared positive. *Anal.* Calcd. for $C_{11}H_{14}N_2Cl_2$: C, 53.89; H, 5.82; N, 11.55. Found: C, 53.51; H, 5.94; N, 11.05.

1-Salicylidene-2,2-bis(2-chloroethyl)hydrazine (XXI)—Prepared by dissolving (II) (0.75 g.), salicylaldehyde (0.4 g.), and AcOK (0.38 g.) in a dilute EtOH solution and heating on a water bath. Pale yellow oil. Yield, 0.8 g. It could not be purified by distillation or through crystalline salt. *Anal.* Calcd. for $C_{11}H_{14}ON_2Cl_2$: N, 10.73. Found: N, 10.67.

1-(α-Methylbenzylidene)-2,2-bis(2-chloroethyl)hydrazine (XXII)—To a dry Et_2O solution of the free base prepared from (II) (0.75 g.), acetophenone (0.6 cc.) was added and the mixture was kept overnight at room temperature. Pale yellow oil (not distilled.). Yield, 0.8 g. *Anal.* Calcd. for $C_{12}H_{16}N_2Cl_2$: N, 10.81. Found: N, 10.73.

1-(*p*-Dimethylaminobenzylidene)-2,2-bis(2-chloroethyl)hydrazine Hydrochloride (XXIII)—A solution of (II) (0.5 g.) in EtOH (3 cc.) was added to a solution of *p*-dimethylaminobenzaldehyde (0.4 g.) in EtOH (3 cc.) containing AcOK (0.3 g.). The whole mixture was refluxed for 30 min. on a boiling water bath. After removing EtOH, the residue was converted into the picrate. Red scales, m.p. 165~166°, from MeOH. *Anal.* Calcd. for $C_{19}H_{22}O_7N_6Cl_2$: C, 44.11; H, 4.29; N, 16.25. Found: C, 44.17: H, 4.15; N, 16.17.

2-[Bis(2-chloroethyl)hydrazono]propionic Acid (XXIV)—A dry Et_2O solution of the free base prepared from (II) (1 g.) was added with pyruvic acid (0.46 g.) and kept standing for 24 hr. at room temperature. Removal of Et_2O gave a pale yellow oil. It was isolated and identified as S-benzylthiuronium salt. White plates, m.p. 147~148° (decomp.), from EtOH. *Anal.* Calcd. for $C_{15}H_{22}O_2N_2Cl_2S$: C, 45.81; H, 5.64; N, 14.25. Found: C, 45.91; H, 5.52; N, 13.98.

N,N-Bis(2-chloroethyl)acetamide (XXV)[7]—Colorless oil, $b.p_{12}$ 150°.

N,N-Bis(2-chloroethyl)chloroacetamide (XXVI)[8]—Colorless oil, $b.p_8$ 167~169°.

Ethyl N,N-Bis(2-chloroethyl)carbamate (XXVII)[8]—Colorless oil, $b.p_7$ 117~120°. *Anal.* Calcd. for $C_7H_{13}O_2NCl_2$: N, 6.55. Found: N, 6.56.

N-Nitroso-bis(2-chloroethyl)amine (XXVIII)[8]—Yellow oil (not distilled). *Anal.* Calcd. for $C_4H_8ON_2Cl_2$: N, 16.38. Found: N, 15.90.

N,N-Bis(2-hydroxyethyl)-*p*-toluenesulfonamide—Obtained by the usual tosylation of diethanolamine. Yield, 70%. White plates, m.p. 101~102°, from H_2O or dil. EtOH. *Anal.* Calcd. for $C_{11}H_{17}O_3NS$: C, 50.95; H, 6.61; N, 5.40. Found: C, 51.08; H, 6.52; N, 5.53.

N,N-Bis(2-chloroethyl)-*p*-toluenesulfonamide (XXIX)—The above amide (8.5 g.) was chlorinated with $SOCl_2$ (7.3 cc.). Yield, 8 g. White needles, m.p. 48~49°, from petr. ether. *Anal.* Calcd. for $C_{11}H_{15}O_2NCl_2S$: C, 44.60; H, 5.10; N, 4.73. Found: C, 44.78; H, 5.07; N, 4.72.

Determination of Cl^- Liberation and Thiosulfate Consumption in $NaHCO_3$-buffered Solution—Titrations were carried out by the procedures described in the preceding report.[9]

7) A. F. Childs, *et al.*: J. Chem. Soc., **1948**, 2174.
8) E. R. H. Jones, W. H. Wilson: *Ibid.*, **1949**, 547.
9) M. Ishidate, *et al.*: This Bulletin, **6**, 164(1958).

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Summary

1,1-Bis(2-chloroethyl)hydrazine and its hydrazide- and hydrazone-type derivatives were prepared and investigated as to their chemical reactivity and anti-tumor activity against the Yoshida sarcoma.

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96. **Hiroshi Igeta**: Syntheses of Pyridazine Derivatives. V.[1] Nitration of 3-Methoxypyridazine 1-Oxide.

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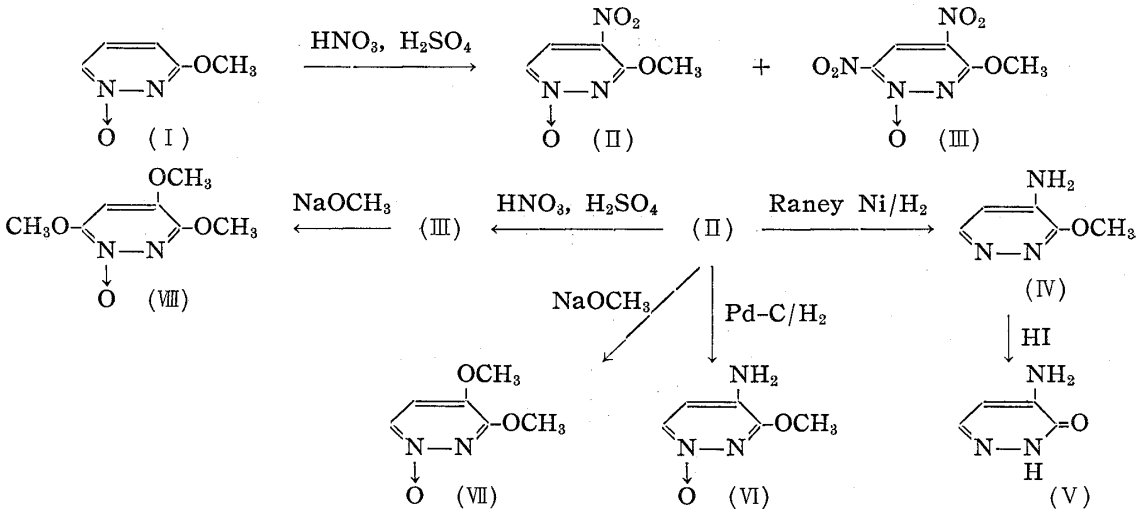
In a previous work,[2] the structure of 3-methoxypyridazine N-oxide was proved to be 3-methoxypyridazine 1-oxide. Nitration of 3-methoxypyridazine 1-oxide (I) was investigated in the present work.

Treatment of this 1-oxide compound (I) with excess of nitric acid in a sulfuric acid solution at 50~55° gave a mononitro compound (II), m.p. 103°, a dinitro compound (III), m.p. 130°, some yellow oils, and a small amount of the starting material.



Catalytic hydrogenation of the mononitro compound (II) with Raney nickel in methanol gave an amino-methoxypyridazine (IV), m.p. 127°, and this was hydrolyzed with hydriodic acid to produce an aminophenol compound (V), m.p. 230°, undepressed on admixture with 4-amino-3-pyridazinol,[3] m.p. 229~230°.[*2] This shows that the nitro group

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*2 This sample was sent from Dr. T. Kuraishi of the University of Nagasaki, to whom the author wishes to express thanks.

1) Part IV: This Bulletin, 8, 368(1960).

2) Part III: *Ibid.*, **7**, 938(1959).

3) T. Kuraishi: *Ibid.*, **6**, 331(1958).