from a benzene solution of the base by the addition of dry HCl, m.p. 189~190° (from methanol). It was soluble in water, hot MeOH and EtOH, and insoluble in benzene and ether. *Anal.* Calcd. for $C_7H_{15}ON_2Cl_3$: C, 33.69; H, 6.05; N, 11.12. Found : C, 33.60; H, 6.40; N, 11.22. Picrate : m.p. 155~157° (from acetone). *Anal.* Calcd. for $C_{13}H_{17}O_8N_5Cl_2$: C, 35.30; H, 3.88; N, 15.83. Found : C, 35.30; H, 4.20; N, 15.52.

N-Bis(β-chloroethyl)alanine (VIII)—A mixture of 50 g. of the hydrochloride of (VII) and 300 cc. conc. HCl was heated at 80° for 1 hr. The reaction product was treated as in the case of (V). m.p. 96~97° (from acetone). It was soluble in water, acetone, and MeOH, sparingly soluble in AcOEt, and insoluble in benzene and ether. *Anal.* Calcd. for $C_6H_{12}O_2NCl_3$: C, 33.56; H, 5.63; N, 5.59. Found : C, 33.55; H, 5.97; N, 5.84. Picrylsulfonate : m.p. 142° (decomp.)(from MeOH). *Anal.* Calcd. for $C_{13}H_{16}O_{11}N_4Cl_2S$: C, 30.78; H, 3.16; N, 11.03. Found : C, 30.78; H, 3.18; N, 10.89.

N-Bis(β-chloroethyl)alanine Ethyl Ester—The hydrochloride of (VIII) was esterified by refluxing with 30% alcoholic HCl, but the ester of this hydrochloride did not crystallize. Picrylsulfonate : m.p. 157~158° (from acetone). *Anal.* Calcd. for $C_{15}H_{20}O_{11}N_4Cl_2S$: C, 33.65; H, 3.76; N, 10.46. Found : C, 33.79; H, 3.72; N, 10.47.

N-Bis(β-hydroxyethyl)taurine (IX)—Into a mixture of 10 g. of taurine, 11 g. of $Ba(OH)_2 \cdot 8H_2O$, and 50 cc. water, 8 g. of ethylene oxide was added, stoppered with a cork, and held at a room temperature for 48 hrs. After removing $Ba^{\cdot\cdot}$ with an approximately equivalent amount of H_2SO_4, the filtrate was condensed *in vacuo* until (IX) began to crystallize out: m.p. 150~151° (from 70% EtOH). *Anal.* Calcd. for $C_6H_{15}O_5NS$: C, 33.77; H, 7.09. Found : C, 33.24; H, 6.73. Yield, 10 g. It was soluble in dil. EtOH and sparingly soluble in abs. EtOH.

N-Bis(β-chloroethyl)taurine—A mixture of 10 g. of (IX) and 60 cc. of $SOCl_2$ was heated at 90~100° on a water bath. After refluxing for 30 mins., the upper layer was removed from the excess $SOCl_2$ by decantation and kept warm for a while under a reduced pressure to remove the accompanying reagent completely. The residue was dissolved in a small quantity of water and added gradually with water. (IX) crystallized from the solution as colorless prisms, m.p. 177~179° (decomp.)(from EtOH). Yield, 8 g. *Anal.* Calcd. for $C_6H_{13}O_3NCl_2S$: C, 28.79; H, 5.24. Found : C, 28.76; H, 5.24.

Summary

N-Bis(β-chloroethyl)-alanine, -glycine, -taurine, and their derivatives were prepared and their properties, toxicity, and effects upon the Yoshida sarcoma of rats were examined. Among these compounds, N-bis(β-chloroethyl)alanine showed the most predominant efficacy by the screening tests and it was presumed that its superiority was at least due in part to the formation of an amphoteric ion in the animal body.

(Received June 21, 1954)

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65. **Michimasa Izumi**[***] : N-Bis(β-chloroethyl)amino Acid N-Oxides and their Effect upon the Yoshida Sarcoma[*].

(*Iatrochemical Institute of the Pharmacological Research Foundation*[**]
and Yoshitomi Pharmaceutical Industries, Ltd.[***])

In the preceeding paper of this series[1)], the amino acid derivatives of nitrogen mustard were shown to have predominant anticancer efficacy. According to previous experiences[2)] that the N-oxide had a more favorable inhibitory effect upon neoplastic growth when they were compared with the original tertiary nitrogen mustards, the N-oxides of N-bis(β-chloroethyl)amino acids were prepared in the hope of getting a still more effective and less toxic chemotherapeutics.

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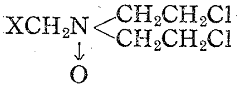
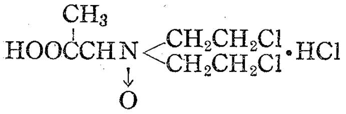
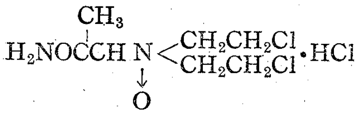
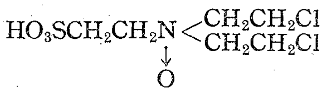
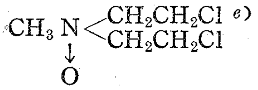
[*] M. Ishidate, Y. Sakurai : Studies on Cancerocidal Substances. XI.
[**] 26, 1-chome, Nishigahara, Kita-ku, Tokyo.
[***] Yoshitomi-machi, Chikujo-gun, Fukuoka-ken (泉 道正).
[1)] M. Izumi : This Bulletin, 2, 275 (1954).

Preparation of N-bis(β-chloroethyl)-glycine N-oxide (I), -alanine N-oxide (II), -taurine N-oxide (III), and their derivatives, together with their toxicity and effect on the Yoshida sarcoma of rats are described in this paper and the results are summarized in Table I.

TABLE I.

Compounds	Free base m.p. °C	Picrate m.p. °C	Picryl-sulfonate m.p. °C	LD_{50} a) mg./kg.	M.E.D. b) mg./kg.	Response of tumor cells c)	Life prolongation of tumor rats d)
 $\mathrm{XCH_2\underset{\overset{\downarrow}{O}}{N}{<}^{CH_2CH_2Cl}_{CH_2CH_2Cl}}$							
X : ·CN·HCl	—	115	—	—	—	/	/
: ·CONH$_2$·HCl	156*)	130	—	30	1	+	/
: ·COOC$_2$H$_5$·HCl	52.5	109	—	55	5	+	—
: ·COOH·HCl	134	—	113	75	5	+	—
: ·COOH	105	—	113			/	/
 $\mathrm{HOOC\overset{CH_3}{\overset{\vert}{C}}H\underset{\overset{\downarrow}{O}}{N}{<}^{CH_2CH_2Cl}_{CH_2CH_2Cl}\cdot HCl}$	110	—	74	55	5	+	/
 $\mathrm{H_2NOC\overset{CH_3}{\overset{\vert}{C}}H\,\underset{\overset{\downarrow}{O}}{N}{<}^{CH_2CH_2Cl}_{CH_2CH_2Cl}\cdot HCl}$	155 (decomp.)	127	—	/	/	/	/
 $\mathrm{HO_3SCH_2CH_2\underset{\overset{\downarrow}{O}}{N}{<}^{CH_2CH_2Cl}_{CH_2CH_2Cl}}$	160 (decomp.)	—	—	300	50	+	/
 $\mathrm{CH_3\,\underset{\overset{\downarrow}{O}}{N}{<}^{CH_2CH_2Cl}_{CH_2CH_2Cl}}$ e)		—	—	80	1	+	+

*) The sample was not perfectly purified.
a) Intraperitoneal dose in rats.
b) Minimum dose which caused a characteristic response in tumor cells.
c) + : Appearance of a definite and characteristic aberration in the nuclei of the tumor cells.
d) + : Half number of the treated tumor rats were alive more than 20 days after transplantation. Life-length of the untreated tumor rat is 10~11 days.
e) The compound is given here as a control for comparison.

Oxidation of the corresponding amine was effected with acceptable yield by the means already reported²⁾. However, the free base (I) or (II) was not stable enough and they tended to liberate Cl^- so promptly in the solution that the hydrochloride, instead of the free base, should be added gradually into the peracid solution containing one molar equivalent of sodium acetate. This improved procedure of oxidation was believed to be useful as the general preparation of N-oxides of nitrogen mustard because the free base was often far more dangerous than its salt in its vesicant activity.

The hydrochlorides of (I) and (II) were readily soluble in water and pH of their 0.5% aqueous solution was about 2. The free base (I) was separated as a crystalline substance by adjusting the concentrated solution of the hydrochloride of (I) to pH 1.8, which was the first free base of nitrogen mustard N-oxide ever obtained in a pure state. The taurine N-oxide (III), which was a stronger acid than (I) or (II), did not form a hydrochloride.

The structures of these N-oxides obtained here were undoubtedly as assumed from the following series of experiments. These N-oxides possessed an oxidizing power similar to methyl-bis(β-chloroethyl)amine N-oxide, since it liberated iodine from a neutral solution of potassium iodide. They were quantitatively reduced to the original tertiary nitrogen

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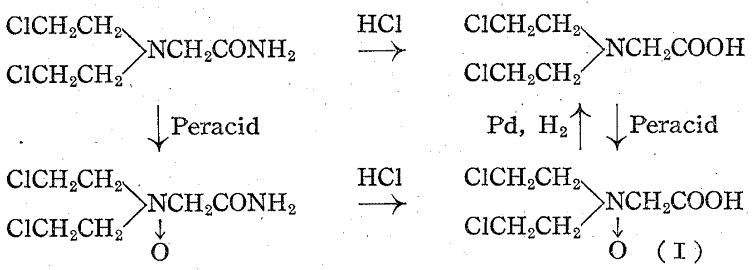
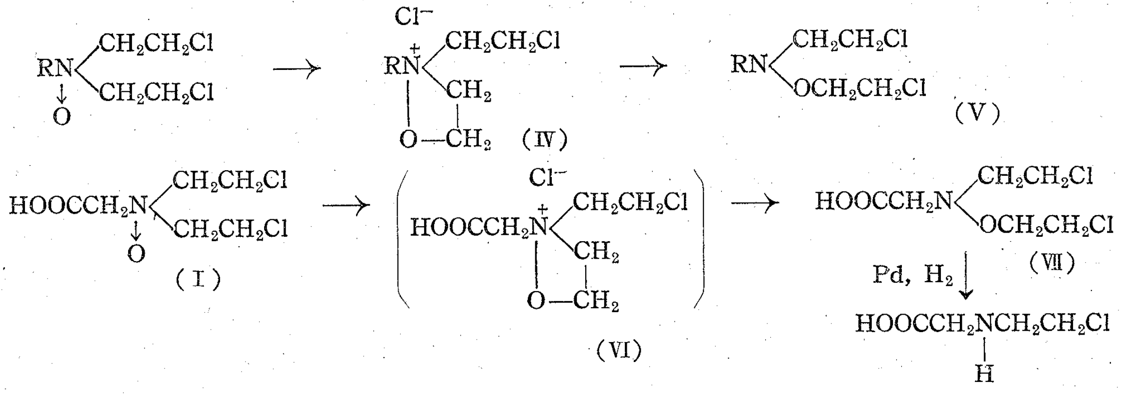
2) I. Aiko, *et al.* : J. Pharm. Soc. Japan, **72**, 1297 (1952); Y. Sakurai, M. Izumi : This Bulletin, **1**, 297 (1953).

mustards by catalytic reduction over palladium-carbon at a room temperature. It was also found that N-bis(β-chloroethyl)aminoacetamide N-oxide could be hydrolysed with conc. hydrochloric acid to an N-oxide which was identical with (I) obtained by the direct oxidation of N-bis(β-chloroethyl)glycine.



Earlier work[3] in this series of study has shown that the nitrogen mustard N-oxides changed to cyclic compounds (IV) in a neutral aqueous solution and it in turn transited to the hydroxylamine derivatives (V). Similar reaction was observed in the case of these N-oxides having a carboxyl group.



When an aqueous solution of the base (I) was heated on boiling water bath for seven minutes, it became turbid and slowly separated an oily substance, which turned crystalline after purification. This product must be N-β-chloroethyl-N-carboxymethyl-O-β-chloroethylhydroxylamine (VII) both from its analytical data and from the fact that it gave N-β-chloroethylglycine by catalytic reduction.

The existence of an intermediate (VI) in this reaction was also presumed from a difference observed in the liberation rate of Cl^- and H^+ of this N-oxide in an aqueous solution, the Rf values on paper chromatogram, and a transition of half-wave reduction potential by polarography in its solution with elapse of time. Details of these experiments will be published in succeeding papers.

The results of the screening test using the Yoshida sarcoma are also shown in Table I. Contrary to our expectation, both chemotherapeutic index and effect on life prolongation of tumor animals were found to be far from improvement when compared with the original parent compounds. No suggestion can be offered here as to this cause, but their mode of action *in vivo* might be somehow different from that of the N-oxides of ordinary nitrogen mustards.

The details on the animal experiments on the compounds described in this paper were reported by Prof. T. Yoshida and others at the Annual Meeting of the Japanese Cancer Association held at Nagoya on April 4, 1954.

The author is grateful to Prof. T. Yoshida for his advices in this study and is also indebted to Messrs. H. Sato, I. Aiko, K. Sawatari, H. Imamura, and K. Suenobu for their collaboration.

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[3] S. Owari: This Bulletin, **1**, 353 (1953); cf. also Footnote 2).

Experimental

N-Bis(β-chloroethyl)alanine N-Oxide (II)—Into a mixture of 60 g. of Ac_2O and 70 g. of 30% H_2O_2, 13 g. of AcONa was dissolved with caution, followed by 40 g. of N-bis(β-chloroethyl)alanine hydrochloride, added in portions into the mixture at 10°. The mixture was kept at the same temperature for 3 hrs., then at 30° for 1 hr. under stirring, and finally held standing overnight at a room temperature. The reaction mixture was strongly acidified with conc. HCl and evaporated to dryness below 30° under a reduced pressure. The residue was extracted with hot acetone and from the acetone extract, the hydrochloride of (II) crystallized when cooled. It was recrystallized from acetone; m.p. 92~93°. It was readily soluble in water, MeOH, and EtOH, less soluble in acetone, sparingly soluble in AcOEt and insoluble in $CHCl_3$ and benzene. Yield, 30 g. *Anal.* Calcd. for $C_7H_{14}O_3NCl_3$: C, 31.54; H, 5.29; N, 5.25. Found : C, 31.56; H, 5.06; N, 5.57.

N-Bis(β-chloroethyl)aminopropioamide N-Oxide—It was obtained by the oxidation of the corresponding amide by the same procedure as that for (II). It was difficult, however, to get crystalline hydrochloride directly from the acetone extract of the residue. It became crystalline first when its pure picrate (m.p. 127°) was treated with HCl and converted again to the hydrochloride, m.p. 155° (decomp.). *Anal.* Calcd. for $C_7H_{15}O_2N_2Cl_3$: C, 31.65; H, 5.69; N, 10.55. Found : C, 31.66; H, 6.04; N, 10.89.

N-Bis(β-chloroethyl)glycine Ethyl Ester N-Oxide—The oxidation product of N-bis(β-chloroethyl)-glycine ethyl ester was obtained as a picrate, m.p. 107° (from EtOH). *Anal.* Calcd. for $C_{14}H_{18}O_{12}N_4Cl_2$: C, 35.54; H, 3.83; N, 11.84. Found : C, 35.81; H, 3.08; N, 12.13. The hydrochloride, m.p. 52.5° (from acetone), was obtained from the picrate. It was soluble in water, MeOH, EtOH, and AcOEt. Picrylsulfonate, m.p. 112~113° (from acetone). *Anal.* Calcd. for $C_{12}H_{14}O_{12}N_4Cl_2S$: C, 28.44; H, 2.96; N, 10.93. Found : C, 28.25; H, 3.01; N, 10.96.

N-Bis(β-chloroethyl)aminoacetonitrile N-Oxide—It was obtained as a picrate, m.p. 115° (from EtOH). *Anal.* Calcd. for $C_{12}H_{13}O_8N_5Cl_2$: C, 33.81; H, 3.07; N, 16.43. Found : C, 33.81; H, 3.03; N, 15.67. A crystalline hydrochloride could not be obtained.

N-Bis(β-chloroethyl)aminoacetamide N-Oxide (IV)—It was obtained as a picrate, m.p. 129~130° (from MeOH). *Anal.* Calcd. for $C_{12}H_{15}O_9N_5Cl_2$: C, 32.45; H, 3.40; N, 15.77. Found : C, 32.69; H, 3.83; N, 15.81. The picrate was converted to the hydrochloride which could not be completely purified, m.p. 156° (washed with dry acetone).

N-Bis(β-chloroethyl)glycine N-Oxide (I)—Oxidation of N-bis(β-chloroethyl)glycine hydrochloride was carried out in the same manner as that of (II). After evaporation of the strongly acidified reaction mixture, the residue was extracted with AcOEt. m.p. 124° (from AcOEt). It was readily soluble in water, acetone, MeOH, and EtOH, soluble in AcOEt, and insoluble in benzene. *Anal.* Calcd. for $C_6H_{12}O_3NCl_3$: C, 28.57; H, 4.79; N, 5.59. Found : C, 28.59; H, 5.03; N, 5.41. The free base of (I) was obtained when 10 g. of (I) hydrochloride was dissolved in 15 cc. of water and this conc. solution was adjusted to pH 1.8 by the addition of sat. soln. of $NaHCO_3$. Rubbing the wall of the flask facilitated crystallization of the base. It was purified by quick recrystallization from EtOH. m.p. 105°(decomp.). *Anal.* Calcd. for $C_6H_{11}O_3NCl_2$: C, 33.32; H, 5.19; N, 6.52. Found : C, 33.25; H, 5.02; N, 6.57.

Hydrolysis of (IV)—Crude hydrochloride of (IV), obtained from its pure picrate, was dissolved in conc. HCl and heated at 80° for 2 hrs. The solution was evaporated to dryness and the residue was extracted with hot AcOEt. m.p. 130° (from AcOEt), showed no depression of melting point when admixed with (I) hydrochloride described above.

Catalytic Reduction of (I)—One gram of (I) hydrochloride was dissolved in 10 cc. MeOH and shaken in a hydrogen stream with Pd (0.1 g.) at a room temperature by which 96 cc. (1 mole) of H_2 was absorbed during 6 mins. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue became crystalline and melted at 74° without further purification. Yield, 0.9 g. It showed no depression of m.p. on admixture with the hydrochloride of N-bis(β-chloroethyl)-glycine.

Catalytic Reduction of (II)—One gram of (II) hydrochloride yielded 0.9 g. of the hydrochloride of N-bis(β-chloroethyl)alanine by the same reduction as above. Absorption of H_2 was 89 cc. (1 mole) in this case and the reduction product melted at 95°, which showed no depression with the authentic sample.

N-β-Chloroethyl-N-carboxymethyl-O-β-chloroethylhydroxylamine (VII)—A solution of 3 g. of the free base of (I) in 6 cc. of water was heated on a boiling water bath for 7 mins. The separated oil was extracted with ether and ether was distillled off. About 1 g. of crude oil was obtained and its solution in 1 cc. of benzene was passed through alumina column. To a portion of the benzene eluate, which was acid to thymol blue, petroleum ether was added and the mixture was kept in a cool place. The hydroxylamine (VII) crystallized gradually; m.p. 50~53°. *Anal.* Calcd. for $C_6H_{11}O_3NCl_2$: C, 33.32; H, 5.13; N, 5.62. Found : C, 33.57; H, 4.82; N, 6.76. It was soluble in ether, benzene, EtOH, acetone and in $NaHCO_3$ solution, and insoluble in water and petroleum ether.

Catalytic Reduction of (VII)—To a solution of 3 g. of (VII) in 20 cc. glacial AcOH, 0.5 g. of $PdCl_2$ dissolved in 5 cc. of conc. HCl was added and the mixture was shaken in H_2 stream at a room temperature for 4 hrs. About 1 mole of H_2 was absorbed. The filtrate from the reaction mixture was distilled under a reduced pressure and the residue was extracted with acetone. A crystalline hydrochloride of N-β-chloroethylglycine separated when the acetone extract was added with ether. It was purified by dissolving it in benzene and adding petroleum ether into the solution. m.p. 53~54°. *Anal.* Calcd. for $C_4H_9O_2NCl_2$: C, 27.59; H, 5.21; N, 8.05. Found: C, 27.75; H, 5.09; N, 8.02.

N-Bis(β-chloroethyl)taurine N-Oxide (III)—Six grams of 30% H_2O_2 was added with caution into 6 g. of Ac_2O. Into this mixture, 5 g. of N-bis(β-chloroethyl)taurine was added in portions and the mixture was kept below 30° for 5 hrs. under stirring. The reaction mixture was filtered if necessary, and the filtrate was evaporated by aspiration under 40°. The crystalline residue obtained here was washed with 20 cc. EtOH and recrystallized from a large quantity of hot EtOH as colorless scales, m.p. 160°(decomp.). Yield, 2 g. It did not form a hydrochloride. It was readily soluble in water but less soluble in EtOH. *Anal.* Calcd. for $C_6H_{13}O_4NCl_2S$: C, 27.07; H, 4.92; N, 5.27. Found: C, 27.35; H, 4.57; N, 5.58.

Summary

N-Oxides of N-bis(β-chloroethyl)-glycine, -alanine, -taurine, and their derivatives were prepared and their chemical properties, toxicity, and effect upon the Yoshida sarcoma of rats were examined. Contrary to our expectations, inhibitory action of these N-oxides against neoplastic growth was found to be inferior than that of N-bis(β-chloroethyl)-amino acids.

(Received June 21, 1954)

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66. **Hirotaka Otomasu**: Studies on Phenazines. VII*. Nitration of Phenazine and its Derivatives.

*(Hoshi College of Pharmacy**)*

There are only few reports on the nitration of phenazine and its derivatives. Claus[1] nitrated phenazine by heating it with a mixture of sulfuric and nitric acids, and claimed to have obtained 2-nitrophenazine. Kehrmann[2] carried out the same reaction but under a rather mild conditions, and obtained 1,3-dinitrophenazine.

On nitration of phenazine mono-N-oxide, Wohl[3] reported the formation of a dinitro compound but he did not determine the positions of the nitro groups.

In this paper, nitration of phenazine and its derivatives under a fixed conditions, i.e. sulfuric acid and potassium nitrate at 0°, is reported.

Phenazine (I) was nitrated with concentrated sulfuric acid and potassium nitrate at 0°, but the reaction did not proceed, recovering the starting material. When reaction temperature was raised to 60°, 1,3-dinitrophenazine (II) was obtained.

From phenazine 5-N-oxide (III), two mono-nitro compounds, m.p. 204° and 213°, were produced in a good yield. The nitro compound of m.p. 204°, the main product, was determined to be 3-nitrophenazine 5-N-oxide (IV), as 1- and 3-positions of phenazine 5-

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* Part VI: This Bulletin, **2**, 53 (1954).

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[1] A. Claus; Ber., 8, 39 (1875).

[2] F. Kehrmann, E. Havas: Ber., **46**, 347 (1913).

[3] A. Wohl: Ber., **36**, 4139 (1903).