233~235° erhalten. $C_{13}H_{11}O_2N_4Cl$ —Ber.: C, 53.70; H, 3.79; N, 19.28. Gef.: C, 53.53; H, 3.87; N, 18.69.

ω, ω' -Dichlor-4-chlor-2,6-lutidin—50 mg ω, ω' -Dioxy-4-chlor-2,6-lutidin, 3 ccm Chloroform und 1 g Thionylchlorid wurden zusammen bei 70~80° 1 Stunde lang erwärmt. Das Lösungsmittel wurde verdampft und das bei der Verdünnung mit Wasser ausgeschiedene Öl mit Petroläther ausgezogen. Die Petroläther-Lösung wurde nach dem Trocknen mit Natriumsulfat vom Lösungsmittel befreit, wobei der Rückstand sich gänzlich erstarrte. Die Ausbeute: 50 mg. Beim Umkristallisieren aus Petroläther bildeten sie Tafeln vom Schmp. 55~56.5°. $C_7H_8NCl_3$ —Ber.: C, 39.90; H, 2.85; N, 6.65. Gef.: C, 40.14; H, 2.59; N, 6.84.

Zusammenfassung

Die Bromierung von α -Picolin, 4-Chlor- α -picolin und 4-Chlor-2,6-lutidin mittels N-Bromsuccinimides wurde versucht. Beim α -Picolin wurde im Laufe der Reaktion ein deutliches Entstehen vom freiem Brom bemerkt und das entsprechende ω -Bromid mit schlechter Ausbeute erhalten. Beim weniger basischen 4-Chlor- α -picolin bzw. 4-Chlor-2,6-lutidin verlief die Reaktion weit glatter, und die Ausbeute von Bromderivaten war besser. Das 4-Chlor- α -picolin gab dabei das ω -Mono- und ω -Dibromid, und aus dem 4-Chlor-2,6-lutidin ergab sich das ω -Mono-, ω, ω - und ω, ω' -Dibromid. Die Trennung dieser Bromderivate wurde auf Grund ihrer verschiedenen Basizität durch Behandlung mit 10 bzw. 20 proz. Bromwasserstoffsäure erzielt. Jedes Bromderivat wurde durch Verseifung in den entsprechenden Alkohol bzw. Aldehyd übergeführt.

(Eingegangen am 30. Juni, 1953)

73. Yoshio Sakurai** and Michimasa Izumi***: N-Oxides of β -Chloroethylamine Derivatives.*

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So many kinds of tertiary β -chloroethylamines have been reported in literatures and discussed as to their cancerocidal activities. Most of them, however, were concluded not to precede to methyl-bis(β -chloroethyl)amine (HN_2) in clinical evaluation.

We had reported in the previous paper¹⁾ that the N-oxide of HN_2 was more effective and less toxic than the corresponding tertiary amines in animal experiments with Yoshida sarcoma rats and this result has been so far confirmed by the clinical experiences which have been carried out in Japan through a year.

We then attempted to synthesize the N-oxides of many kinds of nitrogen mustard in order to compare their efficacy against the Yoshida sarcoma with the corresponding tertiary amines. The compounds prepared in this study are shown in Tables I to III.

TABLE I
Monofunctional Compounds

Compound	Hydrochloride m.p. °C	Picrate m.p. °C	LD ₅₀ mg./kg.	Tumor cell response
1: $(CH_3)_2NCH_2CH_2Cl$	200~201	117~118	30~50	—
2: its oxide	125~127	114~116	300	—
3: $(C_2H_5)_2NCH_2CH_2Cl$	210~211	115~117	30~50	—
4: its oxide	144~146	136~137	400~500	—

* M. Ishidate, Y. Sakurai: Studies on Cancerocidal Substances. IV.

** 33, Nishigahara-machi, Kita-ku, Tokyo (桜井欽夫).

*** Yoshitomi-machi, Chikujo-gun, Fukuoka-ken (泉 道正).

1) I. Aiko, S. Owari, M. Torigoe: J. Pharm. Soc. Japan, 72, 1297(1952).

5:	$\begin{array}{c} \text{CH}_2\text{-CH}_2 \\ \diagup \quad \diagdown \\ \text{O} \quad \text{NCH}_2\text{CH}_2\text{Cl} \\ \diagdown \quad \diagup \\ \text{CH}_2\text{-CH}_2 \end{array}$	180~182		70~100	—
6:	its oxide	146~148	170~172	500	—
7:	$(\text{C}_6\text{H}_5\text{CH}_2)_2\text{NCH}_2\text{CH}_2\text{Cl}$	189		120	—
8:	its oxide	163~164		350	—

TABLE II
Bifunctional Compounds.

Compound	Hydro- chloride m. p. °C	Picrate m. p. °C	LD ₅₀ mg./kg.	Tumor cell response
9: $\text{CH}_3\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	110	133	1.7	+
10: its oxide	109~110	122~124	80	+
11: $\text{C}_2\text{H}_5\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	138~140	98~99	0.5	+
12: its oxide	80~82*)	142	30	+
13: $\text{C}_3\text{H}_7\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	105~111	99	0.8	+
14: its oxide	84~86	115~116	30	+
15: $i\text{-C}_3\text{H}_7\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	214~216	90~95	1.0	±
16: its oxide	121~123	124~126	15	±
17: $\text{C}_4\text{H}_9\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	92~95		1.0	+
18: its oxide	56~58	100~101	1.0	+
19: $\text{C}_5\text{H}_{11}\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	79.5~80.5	75~77	3.0	+
20: its oxide		88~90	75	+
21: $i\text{-C}_5\text{H}_{11}\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	100~101	103	3.0	+
22: its oxide	75~76	114~115	30~40	+
23: $\text{CH}_2=\text{CH}-\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	108~109	121	0.8	+
24: its oxide	80~81	108~110	20	+
25: $\begin{array}{c} \diagup \quad \diagdown \\ \text{H} \quad \text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2 \\ \diagdown \quad \diagup \end{array}$	176~178		3.0	+
26: its dioxide	146~177		75	+
27: $\text{C}_2\text{H}_5\text{-O-C}_2\text{H}_4\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	110~116	87~89	3.0	+
28: its oxide	88~90	128~130	20	+
29: $\text{C}_6\text{H}_5\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	145~147	125~127	10	+
30: its oxide	103~104	124~125**)	35	+
31: $(\text{C}_2\text{H}_5)_2\text{NCH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	141~143	127~129	8.0	+
32: its oxide	125~127	123~125	150	+
33: $\begin{array}{c} \text{CH}_2\text{CH}_2 \quad \text{CH}_2\text{CH}_2 \\ \diagdown \quad \diagup \quad \diagdown \quad \diagup \\ \text{ClCH}_2\text{CH}_2\text{N} \quad \text{NCH}_2\text{CH}_2\text{Cl} \\ \diagup \quad \diagdown \quad \diagup \quad \diagdown \\ \text{CH}_2\text{CH}_2 \quad \text{CH}_2\text{CH}_2 \end{array}$	310~315		0.7	+
34: its dioxide	220~222	170~173	100~150	±

*) m.p. 85~86° according to Bergmann and Stahmann²⁾.

**) See the experimental part of this paper.

TABLE III
Bromo Derivatives and Polyfunctional Compounds.

Compound	Hydro- chloride m. p. °C	Picrate m. p. °C	LD ₅₀ mg./kg.	Tumor cell response
35: $\text{CH}_3\text{N}(\text{CH}_2\text{CH}_2\text{Br})_2$	134~135*)	116~118	8.0	±
36: its oxide	131		2.0	±
37: $\text{ClCH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	130~131	136~137	2.5~3.0	+
38: its oxide	91~92	126~127	3.0	+
39: $(\text{ClCH}_2\text{CH}_2)_2\text{NCH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	113~114		2.0	+
40: its dioxide	147~148	105~106	3.0	+
41: $(\text{ClCH}_2\text{CH}_2)_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$	138	137~138	3.0	+
42: its dioxide	140~141**)	115	0.8	+

* m.p. of hydrobromide.

** m.p. of anhydrous crystals. It melts often at 90° with water of crystallization.

Out of twenty-one oxides in these Tables, Nos. 10, 12, and 38 had already been prepared by Bergmann and Stahmann²⁾, and No. 8 was described by Nickerson and Gump³⁾ as an adrenergic blocking substance. The process of synthesis of tertiary amines and their oxides, which were described in our preceding paper, were in general adopted in this study. In cases of the preparation of higher homologs of alkyldiethanolamines, condensation of alkyl halide with diethanolamine is preferable to that of alkylamine with ethylene oxide, and the chlorination was accomplished with thionyl chloride in benzene solution without difficulty.

For oxidation of the amines, we found it better to add the base little by little into the mixture of aqueous hydrogen peroxide and acetic anhydride, instead of adding acetic anhydride into base-hydrogen peroxide mixture. Aryl-bis(β -chloroethyl)amines, e.g. phenyl- or β -naphthyl nitrogen mustard, were the exceptional cases, in which nothing but a strongly stained product was obtained.

Most of the hydrochlorides of the N-oxides are crystalline but are somewhat hygroscopic substances, and easily soluble in water, slightly soluble in acetone. Free bases of the N-oxides have in general very hydrophilic character contrary to the corresponding tertiary amines, which are sparingly soluble in water. Solubility of the free base decreases when alkyl group becomes larger or is substituted by aralkyl group such as benzyl. They are all strongly oxidative and have the characteristic half-wave reduction potentials in polarographic determination^{*)}. They undergo intramolecular transformation into the oximine-ring intermediates just like the case of methyl-bis(β -chloroethyl)amine N-oxide with successive change to the trisubstituted hydroxylamine derivatives^{**)}.

Concerning the biological activities and toxicities of the compounds, procedures had been reported by one of us⁴⁾ and the further detail will be published elsewhere. In short, the result was as follows, so far as the experiment with Yoshida sarcoma rats is concerned: 1) Necessity of polyfunctionality of the molecule in order to be effective in cancer retardation is also true without exception in the case of N-oxides: 2) Decrease in toxicity is generally far more dominant than decrease in effectiveness when the tertiary amines are changed to the N-oxides. Accordingly the chemotherapeutic index is remarkably improved with N-oxide formation. 3) Tri- or tetra-functional N-oxides are not less toxic than the corresponding amines. The fact is also observed in the case of methyl-bis(β -bromoethyl)amine N-oxide. Compounds No. 10 and 22 are now under clinical investigations.

We wish to acknowledge with thanks Prof. Tomizo Yoshida and Messrs. Hiroshi Sato, Eiichi Matsui, Hiroshi Imamura, and Takeo Namba, by whom the animal experiments in this work were carried out.

Experimental

Preparation of alkyl-bis(β -hydroxyethyl)amines—a) 2 mol. of ethylene oxide was passed into the aqueous solution of one mole of the amine (about 10~15%) with mechanical stirrer and chilling. After the reaction ended, the solution was fractionated in vacuo. In such a case as it happened to yield mono-substituted amine as a by-product, it was often preferable not to keep the reaction temperature too low and to make stirring more effective.

b) A mixture of one mole each of alkyl halide and diethanolamine was added with 1.2~1.3 moles of anhydrous K_2CO_3 and refluxed for 2~8 hrs., without a solvent in an oil bath. After cooling, a proper quantity of alcohol was added and the insoluble inorganic salts were filtered off. The filtrate was subjected to vacuum distillation. Yield was satisfactory in most cases.

Halogenation of β -hydroxyethyl derivatives—Simpler derivatives were easily chlorinated by refluxing with thionyl chloride without a solvent for 0.5~2 hrs., but their higher homologs were

2) M. Bergmann, M. A. Stahmann: J. Org. Chem., 11, 586 (1946).

3) M. Nickerson, W. S. Gump: J. Pharmacol. & Exptl. Therap., 97, 25 (1949).

* ** The details will be published in our succeeding papers.

4) M. Ishidate, T. Yoshida, Y. Sakurai, H. Sato, K. Kobayashi: "Gann", 43, 171 (1952): A part of the work was also reported at the Annual Meeting of the Japanese Cancer Association at Tokyo, April, 1953.

frequently subdued to partial decomposition and darkening. To use benzene as diluting solvent is preferable in such an instance. Thionyl chloride for this purpose must be extremely pure and the reaction temperature should be kept at the most under 80°.

Preparation of N-oxides—Oxidation was accomplished by many kinds of processes, e.g. by using hydrogen peroxide or peracids. We found, however, that the following procedures were satisfactory in most of our experiments.

Into 1 mole of ice-chilled acetic anhydride was added, in drops, 1 mole of hydrogen peroxide as a 30% aqueous solution, under vigorous stirring. After becoming homogeneous, the benzene solution of the free base of the tertiary amine was dropped in. Reaction is exothermic and fall of the temperature means a completion of the oxidation. The mixture was heated cautiously to 30~40° at the end of the reaction, if necessary. Stirring should be continued throughout the reaction. The reaction mixture was strongly acidified with hydrochloric acid and evaporated to dryness in vacuo. The residue became solid or crystalline and could be recrystallized from acetone, benzene, or acetone-benzene mixture. In case of N-oxides, hydrochloride of which has little inclination to crystallize, it is advantageous to separate it once as a picrate and regenerate the hydrochloride from the purified picrate. Yield was over 60% in some cases, but in general 20~50% in most of the experiments. An example of the practice is shown in case of the compound No. 40.

Dimethyl- β -chloroethylamine N-oxide—Hydrochloride: Colorless and hygroscopic prisms, m.p. 125~127°. Yield, 20%. *Anal.* Calcd. for $C_4H_{11}ONCl_2$: C, 30.00; H, 6.91; N, 8.75. Found: C, 30.02; H, 7.16; N, 8.61. Picrate, m.p. 114~116°.

Diethyl- β -chloroethylamine N-oxide—Hydrochloride crystallized from acetone as very hygroscopic and colorless prisms, m.p. 144~146°. Yield, 50%. *Anal.* Calcd. for $C_6H_{15}ONCl_2$: C, 38.29; H, 7.97; N, 7.44. Found: C, 38.45; H, 7.04; N, 7.65. Picrate: m.p. 136~137°. *Anal.* Calcd. for $C_{12}H_{17}O_8N_4Cl$: C, 37.89; H, 4.47. Found: C, 37.42; H, 4.02.

N- β -Chloroethylmorpholine N-oxide—Hydrochloride, colorless plates as crystallized from alcohol-acetone mixture. Yield, 65%. *Anal.* Calcd. for $C_6H_{13}O_2NCl_2$: C, 35.64; H, 6.44; N, 6.93. Found: C, 35.71; H, 6.13; N, 6.88. Picrate, m.p. 170~172°.

Dibenamine N-oxide—Hydrochloride, colorless scales, m.p. 163~164°(decomp.). Yield, 60%. *Anal.* Calcd. for $C_{16}H_{19}ONCl_2$: C, 61.52; H, 6.14. Found: C, 61.54; H, 6.10. The free base is soluble in benzene.

Ethyl-bis(β -chloroethyl)amine N-oxide—Hydrochloride, colorless and very hygroscopic prisms from acetone-ether mixture, m.p. 80~82°. *Anal.* Calcd. for $C_6H_{14}ONCl_3$: C, 32.43; H, 6.30. Found: C, 32.20; H, 6.62. Picrate, m.p. 142°(decomp.).

N-Propyl-bis(β -chloroethyl)amine N-oxide—Hydrochloride, colorless prisms from dry acetone, m.p. 84~86°. Yield, 20%. Picrate, m.p. 115~116°. *Anal.* Calcd. for $C_{13}H_{18}N_4O_8Cl_2$: C, 36.38; H, 4.19. Found: C, 36.92; H, 4.81.

Isopropyl-bis(β -chloroethyl)amine N-oxide—Hydrochloride, well crystallizing and non-hygroscopic prisms from acetone, m.p. 121~123°. Yield, 65%. *Anal.* Calcd. for $C_7H_{16}ONCl_3$: C, 35.59; H, 6.77. Found: C, 35.71; H, 7.14. Picrate, m.p. 124~126°. *Anal.* Calcd. for $C_{13}H_{18}O_8N_4Cl_2$: C, 36.38; H, 4.19. Found: C, 36.27; H, 3.99. Picrate, m.p. 100~101°.

Butyl-bis(β -chloroethyl)amine N-oxide—Hydrochloride melted at about 56~58°, but could not be obtained as an analytically pure preparation.

Amyl-bis(β -chloroethyl)amine N-oxide—Hydrochloride, light yellow viscid syrup, which gave yellow picrate melting at 88~90°. *Anal.* Calcd. for $C_{15}H_{22}O_8N_4Cl_2$: C, 39.40; H, 4.85. Found: C, 39.16; H, 4.77. Animal test was carried out with syrupy hydrochloride generated from purified picrate. Yield of Picrate, 45%.

Isoamyl-bis(β -chloroethyl)amine N-oxide—Hydrochloride was obtained as colorless scales, m.p. 75~76°, when dissolved in dry acetone and reprecipitated by dry ether. *Anal.* Calcd. for $C_9H_{20}ONCl_3$: C, 40.38; H, 7.61. Found: C, 40.60; H, 7.41. Picrate, yellow needles, m.p. 114~115° (decomp.). *Anal.* Calcd. for $C_{15}H_{22}O_8N_4Cl_2$: C, 39.38; H, 4.85. Found: C, 39.10; H, 4.71. Yield of picrate, 50%. The free base was slightly soluble in benzene.

Allyl-bis(β -chloroethyl)amine N-oxide—Hydrochloride was dissolved in dry acetone and reprecipitated with dry ether. Colorless prisms, m.p. 80~81°. Very unstable substance and colors when heated over 40°. Yield, very poor (ca. 5%). *Anal.* Calcd. for $C_7H_{14}ONCl_3$: C, 35.84; H, 6.01. Found: C 34.96; H, 5.77. Picrate from alcohol, m.p. 108~110°. *Anal.* Calcd. for $C_{13}H_{18}O_8N_4Cl_3$: C, 36.41; H, 3.78. Found: C, 36.14; H, 4.34.

Cyclohexyl-bis(β -chloroethyl)amine N-oxide—Hydrochloride, colorless plates from alcohol-acetone-benzene mixture, m.p. 146~147°. *Anal.* Calcd. for $C_{16}H_{20}ONCl_3$: C, 43.41; H, 7.29. Found: C, 43.14; H, 7.26. Yield, 70%.

Ethoxyethyl-bis(β -chloroethyl)amine N-oxide—Hydrochloride melted at about 88~90°, but it was difficult to analyze because of its extremely hygroscopic character. Picrate, yellow needles, m.p. 125~126°. *Anal.* Calcd. for $C_{14}H_{21}N_4O_9Cl_2$: C, 36.61; H, 4.40. Found: C, 36.40; H, 4.29. Yield of picrate, 66%.

Benzyl-bis(β -chloroethyl)amine N-oxide—Hydrochloride melted at 103~104° immediately after recrystallization from acetone-toluene mixture (1:1), but m.p. changed to 114~115° after a few days. Yield, 70%. *Anal.* Calcd. for $C_{11}H_{16}NOCl_3$ (m.p. 114~115°): C, 46.39; H, 5.67. Found: C, 46.45; H, 5.42. Picrate, yellow needles, m.p. 124~125°(decomp.). *Anal.* Calcd. for $C_{17}H_{18}O_8N_4Cl_2$: C, 42.86; H, 3.77. Found: C, 42.97; H, 3.98.

Diethylaminoethyl-bis(β -chloroethyl)amine N-oxide—Crude dihydrochloride was dissolved in abs. alcohol, shaken with active carbon, and the filtrate was added with dry ether in drops. Colorless prisms separated, m.p. 125~127°(decomp.). Yield, 15%. Dipicrate, from alcohol-acetone mixture, m.p. 123~125°(decomp.). *Anal.* Calcd. for $C_{22}H_{28}O_{10}N_8Cl_2$: C, 36.12; H, 3.86. Found: C, 35.91; H, 3.60.

N,N-bis(β -Chloroethyl)piperazine N-oxide—Dihydrochloride, colorless needles, m.p. 220~222°. *Anal.* Calcd. for $C_8H_{18}O_2N_2Cl_4$: C, 30.33; H, 5.69; N, 8.86. Found: C, 30.21; H, 5.72; N, 8.88. Dipicrate, m.p. 170~173°.

Methyl-bis(β -bromoethyl)amine N-oxide—To prepare the free base solution of the tertiary amine, excess of alkali should be avoided because it tends to transform into ethyleneimonium ion faster than the corresponding chloro derivative. Oxidation was carried out very cautiously under 40° but the reaction mixture still intensively darkened and yield of the oxide was not more than 5%. Hydrochloride, colorless plates from acetone, m.p. 131°. *Anal.* Calcd. for $C_5H_{12}ONBr_2Cl$: C, 20.17; H, 4.07. Found: C, 20.19; H, 4.25.

Tris(β -chloroethyl)amine N-oxide—Hydrochloride, colorless prisms from acetone, m.p. 91~92°. *Anal.* Calcd. for $C_9H_{15}ONCl_3$: C, 28.23; H, 5.09; N, 5.49. Found: C, 27.99; H, 5.20; N, 5.21. Picrate, m.p. 126~127°.

Tetrakis(β -chloroethyl)ethylenediamine N,N'-dioxide—50 g. of N,N'-tetrakis(β -chloroethyl)-ethylenediamine hydrochloride, m.p. 113°, was dissolved in water (50 cc.). Just enough quantity of Na_2CO_3 to liberate the free base was added to the solution and the free base was taken up in benzene. Into a mixture of acetic anhydride and hydrogen peroxide, which was preliminarily prepared and chilled, the benzene solution of the base was added by drops during 0.5 hr. and stirring continued for 3 hrs., during which the temperature was kept under 30°. Then 200 cc. of 10% HCl was added and a small quantity of oily substance that separated was extracted by ether. The aqueous solution was evaporated to dryness in vacuo and the crystalline residue was recrystallized from 90% methanol. Dihydrochloride: Colorless scales, m.p. 147~148°. Yield, 25%. *Anal.* Calcd. for $C_{10}H_{22}O_2N_2Cl_6$: C, 28.94; H, 5.34. Found: C, 29.02; H, 5.90. Dipicrate: Yellow prisms, m.p. 105~106°(decomp.). *Anal.* Calcd. for $C_{22}H_{26}O_{10}N_8Cl_4$: C, 33.01; H, 3.27. Found: C, 32.67; H, 3.23.

Tetrakis(β -chloroethyl)propylenediamine N,N'-dioxide—Dihydrochloride was crystallized from acetone as a monohydrate, m.p. 90°. It lost water of crystallization in vacuum desiccator with P_2O_5 and melted at 140~141°. Colorless needles. *Anal.* Calcd. for $C_{11}H_{24}O_2N_2Cl_6$: C, 30.77; H, 5.64. Found: C, 30.48; H, 5.64. Dipicrate, yellow needles, m.p. 115°(deomp.). *Anal.* Calcd. for $C_{23}H_{28}O_{10}N_8Cl_4$: C, 33.90; H, 3.47. Found: C, 34.18; H, 3.62. Yield of picrate, 80%.

Summary

Twenty N-oxide derivatives of nitrogen mustards, including seventeen new compounds, were synthesized. Their toxicities were compared with those of the corresponding tertiary amines and they were also tested as to their cancer retarding effects using the Yoshida ascites sarcoma of rats.

(Received June 30, 1953)