40.04; H, 4.37; N, 15.20. The hydrochloride became finally crystalline when it was prepared from its purified picrate, as colorless and hygroscopic needles, m.p. 182~183°. *Anal.* Calcd. for $C_{12}H_{28}N_2Cl_4$: C, 42.12; H, 8.25; N, 8.19. Found: C, 41.95; H, 8.31; N, 8.35.

N,N′-Bis(2-chloroethyl)-N,N′-dimethylnonamethylenediamine dipicrate (from EtOH): m.p. 147~148°. Yield, 70%. *Anal.* Calcd. for $C_{27}H_{38}O_{14}N_8Cl_2$: C, 42.13; H, 4.94; N, 14.56. Found: C, 42.42; H, 5.02; N, 14.57. The hydrochloride was prepared from the purified picrate as colorless and hygroscopic needles, m.p. 161° (from Me_2CO-EtOH). *Anal.* Calcd. for $C_{15}H_{34}N_2Cl_4$: C, 46.87; H, 8.85; N, 7.29. Found: C, 46.83; H, 8.77; N, 7.11.

Bis[(N-2-chloroethyl-N-methyl)aminoethyl] ether: In this case, the hydroxy compound should be kept with $SOCl_2$ at room temperature without any solvent for 1 day, otherwise the ether bond is cleaved and N-methyl-bis(2-chloroethyl)amine alone is formed. The picrate of the product could be purified by recrystallization from Me_2CO of the crude picrate obtained from the reaction mixture. m.p. 175°. Yield, a few %. Hydrochloride could not be obtained in crystalline state.

Determination of Cl^- and H^+ Liberation—An aq. solution of hydrochloride of the base (0.02 m. mole/cc.) was neutralized with 2 mol. equiv. of NaOH and incubated at 37°. An aliquot of the solution was successively taken out at every 1-hr. intervals. Cl^- and H^+ were titrated with 0.01*N* $AgNO_3$(indicator: Na-salt of tetrabromophenolphthalein ethyl ester) and 0.01*N* NaOH(indicator, thymolphthalein) respectively.

Determination of Cl^- in $NaHCO_3$-buffered Solution—A sample solution of the same concentration as above, but containing 0.08 m.mole/cc. of $NaHCO_3$, was titrated with 0.01*N* $AgNO_3$ by the same procedure as described above.

Determination of Thiosulfate Consumption in $NaHCO_3$-buffered Solution—The sample (0.5 m. mole) was added with 10% aq. $NaHCO_3$ (2 cc.) and 0.1*N* $Na_2S_2O_3$ (10 cc.), and immediately diluted to 50 cc. with H_2O. It was incubated at 37° and 10-cc. portion was taken out after 10 mins., 2 hrs., and 24 hrs., if necessary from the beginning of incubation, and titrated with 0.02*N* I_2.

Determination of Rate of Viscosity Depression of Polymetacrylate Solution—Experiment was carried out after the description published by one of the authors.[3]

Summary

Preparation, chemical properties, and carcinostatic activity on the Yoshida sarcoma of N,N′-bis(2-chloroethyl)-N,N′-dimethylpolymethylenediamines were discussed. It was concluded that the derivatives of hexamethylenediamine and nonamethylenediamine were more promising than the corresponding lower homologs with respect to chemotherapeutic indices.

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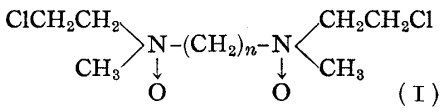
28. Morizo Ishidate, Yoshio Sakurai, and Kozo Maruyama: Studies on Carcinostatic Substances. XIX.* The Preparation and Properties of N,N′-Bis(2-chloroethyl)-N,N′-dimethylpolymethylenediamine N,N′-Dioxides.

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

In the preceding paper, the authors reported on some derivatives of N,N′-bis(2-chloroethyl)-N,N′-dimethylpolymethylenediamine. The present report deals with the preparation of the N,N′-dioxides (I) of those compounds.



(I) n=2, 4, 6, 9

The ordinary oxidation process using peracids was successful in most of the cases.

* Part XVIII: This Bulletin, **6**, 164(1958).

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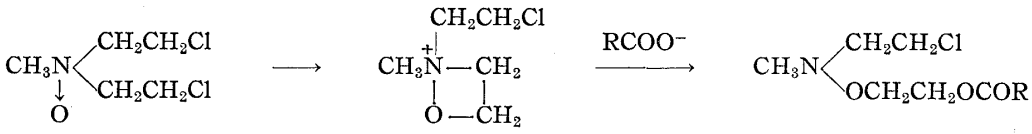
These N,N′-dioxides were however more unstable if anything and there were some difficulties in their purification.

In 1953 the author[1] presented a transformation process of nitrogen mustard N-oxides in a dilute aqueous solution and pointed out that the final stage of this reaction was deemed to be a kind of alkylation of acid residues, viz. carboxylic, phosphoric, and sulfonic acids.



A main object of our study was to find whether the known characteristic reaction of tertiary 2-chloroethylamine N-oxides *in vitro* could take part in exhibiting an anti-cancer activity of these compounds *in vivo* through this transformation reaction.

It has however been proved by one of the authors that the mode of alkylation by such a reaction of the ordinary bifunctional nitrogen mustard N-oxide was of mono-functional character. In order to make the reaction bifunctional, two 2-chloroethyl groups of a compound should be attached to each of the two different nitrogens such as in compounds herein presented.

The chemical reactions of these N,N′-dioxides were first investigated. The titration of Cl^- and H^+, liberating gradually from the bases when their dilute aqueous solutions were kept at 37° for some period, proved that the two kinds of liberation did not proceed in parallel with each other and a delay of H^+ over Cl^--liberation even after 24 hours of incubation should have been induced by the formation of a quaternary base in the solution and not by a simple hydrolysis as shown in Fig. 1.

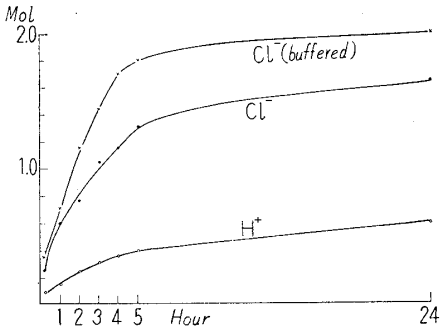
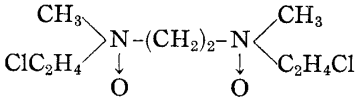


Fig. 1.



The final transformation to the hydroxylamine derivative, however, was not proved, either by titrations or by an analytical survey of an extract of the reaction mixture.

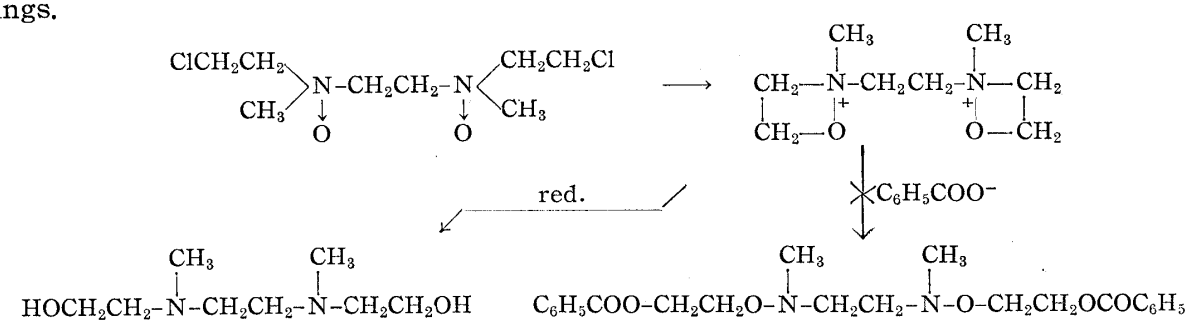
In an earlier experience, it was found that this final transformation product of methyl-bis(2-chloroethyl)amine N-oxide could be more easily recognized if an excess of sodium benzoate was added into the reaction solution, because the less soluble N-(2-chloroethyl)-N-methyl-O-benzoyloxyethylhydroxylamine thus formed could be easily separated from an aqueous layer. However, as will be described in the experimental part, when N,N′-bis(2-chloroethyl)-N,N′-dimethylethylenediamine was dissolved in water with or without an excess of sodium benzoate, no ether-soluble amine was identified.

The reaction mixture with benzoate was continuously extracted for 24 hours with ether in order to promote the transformation, by removing the expected final product, because only the hydroxylamine derivative among the amines existing in the solution would be transferred into the ether extract. However, no amine-like substance was detected in the residue remaining after evaporation of the ether extract and the bicyclic intermediate alone, viz. 2,2′-ethylene-bis(2-methyl-1,2-oxazetidinium) base, was

1) S. Owari : This Bulletin, **1**, 353(1953).

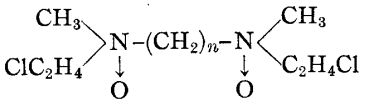
obtained as its picrylsulfonate from the aqueous layer in a good yield.

From this fact, it was deemed improbable that the characteristic transformation reaction might contribute, at least in part, to the exhibition of carcinostatic activity of these compounds. Moreover, these bifunctional di-N-oxides were completely inactivated if they were reduced after transformation *in vivo* into the corresponding cyclic intermediates, because they lost all of their reactive chlorines by reductive cleavage of the rings.



The anti-cancer action of the compounds (I) on the Yoshida sarcoma was observed to be far less potent than the corresponding tertiary amines discussed in the preceding report, as can be seen in Table I.

Table I.



n	MTD mg./kg.	MED mg./kg.	LD_{50} mg./kg.	C.E.
2	100	10	150	+
4	200	10	350	+
6	500	100	750	+
9	50		75	−

It is true that the lower homologs seemed to be still somewhat effective, but the highest homolog ($n=9$) hardly maintained its activity. A major portion of the active homolog changed into the intermediate of transformation instantly after administration and was then reduced to inactive chlorine-free compound, but some amount of the compound, small as it was, showed biological activity, yielding the corresponding tertiary amine by reduction before its transformation reaction began to start. The biological inactivity of transformed intermediate of these compounds was also clearly demonstrated by the fact that an analytically pure sample of 2,2′-ethylene-bis(2-methyl-1,2-oxazetidinium) dipicrylsulfonate caused no morphological aberration on the nuclei or cytoplasma of Yoshida sarcoma cells when it was subjected to the so-called *in vitro* test[2] with the Yoshida sarcoma even in a concentration of 100 μg./cc.[3] With this test method, a detectable minimum concentration of N-methyl-bis(2-chloroethyl)amine N-oxide hydrochloride was determined to be 0.05 μg./cc. In the case of the inactive compound ($n=9$), it is supposed that, on account of the extreme stability of the cyclic intermediate, the transformation reaction proceeds so fast that a sufficient amount of the original N-oxide does not remain in the tissue to show biological activity after it was injected. In conclusion, the N-oxide of this type of structure was deemed to be not promising as carcinostatic agents.

The authors are grateful to Prof. T. Yoshida of the University of Tokyo and to Dr. H. Satoh of Iatrochemical Institute of Pharmacological Research Foundation, Tokyo, for their collaboration in the field of biological experiments. The authors are also indebted to Mr. D. Ohata for carrying out the microanalysis. This work was supported by a Grant in Aid of Scientific Research from the Ministry of Education to which the authors are deeply indebted.

2) M. Ishidate, *et al.*: Gann, **47**, 382(1956).
3) The experiment was carried out by Miss H. Komai of the Iatrochemical Institute.

Experimental

Oxidation of N,N′-Bis(2-chloroethyl)-N,N′-dimethylpolymethylenediamine N,N′-Dioxide—N,N′-Bis(2-chloroethyl)-N,N′-dimethylpolymethylenediamine (6 m. mol.) was dissolved in Et_2O (5 cc.) and added with 2.7% H_2O_2–Et_2O solution. Ac_2O (2.8 cc.) was dropped into the mixture under vigorous stirring and, after 40 mins., it was shaken with 10% HCl (10 cc.). The aqueous layer was separated, washed once with fresh ether, evaporated, and exsiccated *in vacuo*. From the residues, picrates were obtained and purified by recrystallization from Me_2CO–EtOH. Yield, ca. 30% in most cases. Hydrochlorides were very hygroscopic and could not be obtained in crystalline state without a preliminary purification as picrates.

N,N′-Bis(2-chloroethyl)-N,N′-dimethylethylenediamine N,N′-dioxide : Picrate : m.p. 126°(decomp.). *Anal.* Calcd. for $C_{20}H_{24}O_{16}N_8Cl_2$: C, 34.13; H, 3.41; N, 15.93. Found : C, 34.14; H, 3.56; N, 16.04. Hydrochloride : m.p. 149°(decomp.) (from Me_2CO). *Anal.* Calcd. for $C_8H_{20}O_2N_2Cl_4$: C, 30.18; H, 6.28; N, 8.80. Found : C, 30.33; H, 6.44; N, 8.94.

N,N′-Bis(2-chloroethyl)-N,N′-dimethyltetramethylenediamine : Picrate : m.p. 159°. *Anal.* Calcd. for $C_{22}H_{28}O_{16}N_8Cl_2$: C, 36.11; H, 3.83; N, 15.32. Found : C, 36.25; H, 3.94; N, 15.20. Hydrochloride : m.p. 163°(from Me_2CO). *Anal.* Calcd. for $C_{10}H_{24}O_2N_2Cl_4$: C, 34.68; H 6.93; N, 8.09. Found : C, 34.70; H, 7.21; N, 8.05.

N,N′-Bis(2-chloroethyl)-N,N′-dimethylhexamethylenediamine N,N′-dioxide : Picrate m.p. 157°. *Anal.* Calcd. for $C_{24}H_{32}O_{16}N_8Cl_2$: C, 37.94; H, 4.21; N, 14.75. Found : C, 38.41; H, 4.32; N, 14.86. Hydrochloride : m.p. 139°(from Me_2CO). *Anal.* Calcd. for $C_{12}H_{28}O_2N_2Cl_4$: C, 38.50; H, 7.48; N, 7.48. Found : C, 38.19; H, 7.83; N, 7.62.

N,N′-Bis(2-chloroethyl)-N,N′-dimethylnonamethylenediamine N,N′-dioxide : Picrylsulfonate : Colorless needles, m.p. 93°(from H_2O). *Anal.* Calcd. for $C_{27}H_{38}O_{20}N_8Cl_2S_2$: C, 34.87; H, 4.09; N, 12.05. Found : C, 34.95; H, 4.46; N, 12.15. The hydrochloride and picrate were not obtained in crystalline state. The hydrochloride, which was obtained as an exsiccated syrupy residue from the aqueous layer of the reaction mixture, developed one spot on paper chromatogram and was believed to be fairly pure. This was used for animal experiment.

Determination of Cl^-- and H^+-Liberation in Unbuffered or $NaHCO_3$-buffered Solution—Titrations were carried out by the procedures completely analogous to those described by the authors in the preceding report.*

Reaction between N,N′-Bis(2-chloroethyl)-N,N′-dimethylethylenediamine N,N′-Dioxide and Sodium Benzoate in Dilute Aq. Solution—Ten cc. of an aq. solution of the base of N,N′-bis(2-chloroethyl)-N,N′-dimethylethylenediamine N,N′-dioxide (0.02 m.mole/cc.), which was prepared from the hydrochloride solution with Ag_2CO_3, was added with 4 times the equivalent amount of Na benzoate and kept for 24 hrs. at a room temperature with automatic extraction with Et_2O. Nothing was found in the Et_2O extract after being washed with $NaHCO_3$ solution. From the aq. layer in the extracting apparatus, an unknown amine was obtained as a picrylsulfonate, which was recrystallized from Me_2CO. It did not decompose below 300°. The analytical data agreed well with those for 2,2′-ethylene-bis(2-methyl-1,2-oxazetidinium) dipicrylsulfonate. *Anal.* Calcd. for $C_{20}H_{22}O_{20}N_8S_2$: C, 31.66; H, 2.90; N, 14.77. Found : C, 32.01; H. 2.51; N, 15.07.

Summary

Preparation of N,N′-bis(2-chloroethyl)-N,N′-dimethylpolymethylenediamine N,N′-dioxides was described and the relation between the process of characteristic transformation reaction and biological action of these compounds was discussed.

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