(decomp.). No depression of melting point was observed with the authentic specimen of tosyl-cystine.

7) Paper chromatograms—Materials used: Sulfate of (VIII), sulfate of (IX), Reaction Products A and B (q.v.). Solvents used: (a) A ternary mixture from the less hydrated phase obtained from butyl alcohol (80 cc.), acetic acid (20 cc.), and water (100 cc.). (b) A mixture from less hydrated phase obtained from butyl alcohol (60 cc.), phenol (30 cc.), and 0.5% ammonia water (24 cc.). The chromatograms were run for 18 hrs. (the former solvent), or 4 hrs. (the latter solvent) at 21~22°. The paper strips were dried and sprayed with 0.2% aqueous ninhydrin solution and heated at 90~95° for 10 mins. After developing, some of the strips were divided longitudinally into two parts and one was sprayed with Dragendorf's reagent and the other, with ninhydrin. Results of the experiment are summarised in Table II.

Summary

Cysteine up-take of methyl-bis(β-chloroethyl)amine N-oxide (HN_2 N-oxide) in neutral aqueous medium was compared with those of tertiary or N-oxide-form nitrogen mustards. The results suggest that HN_2 N-oxide is reduced to HN_2 and partially to chlorhydrin of HN_2 in such a reducing medium, probably even *in vivo*.

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86. Sakahiko Owari: Transformation Reaction of Nitrogen Mustard N-Oxides in Aqeous Solution.*

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It has been reported earlier[1] that methyl-bis(β-chloroethyl)amine N-oxide (HN_2 N-oxide) tends to transform into the ring-oxide, i.e. N,N-methylchloroethyldimethylene-1,2-oximinium chloride in neutral aqueous solution, which changes successively to N,N-methyl-β-chloroethyl-O-chloroethylhydroxylamine when kept for longer periods under the same conditions. Some nitrogen mustard N-oxides, prepared in order to test their retarding effects[2] on Yoshida sarcoma, were examined in this study as to their tendencies to these transformation reactions. To determine the velocity of ring-formation, titration of liberated Cl^- and H^+ of the free base solution of the compounds at 37° was carried out.

The results are summarized in Figs. 1 and 2, the former showing the curves of the monofunctional compounds and the latter, those of the bifunctional ones.

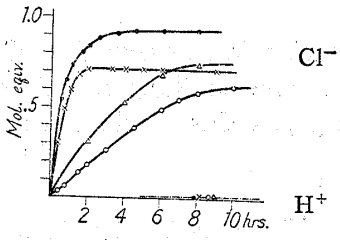

Fig. 1.
- •: Diethyl-β-chloroethylamine N-oxide
- ×: N-β-Chloroethylmorpholine N-oxide
- △: Dibenzyl-β-chloroethylamine N-oxide
- ○: Dimethyl-β-chloroethylamine N-oxide
- ——: Cl^--liberation

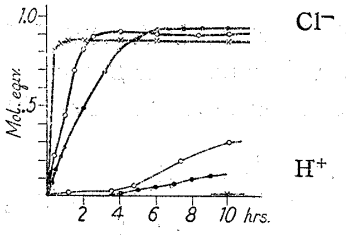

Fig. 2.
- •: N-Methyl-bis(β-chloroethyl)amine N-oxide
- ○: N-Benzyl-bis(β-chloroethyl)amine N-oxide
- ×: N-Isoamyl-bis(β-chloroethyl)amine N-oxide
- —•—•—•—: H^+-liberation

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* M. Ishidate, Y. Sakurai; Studies on Cancerocidal Substances. VIII.

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

2) Y. Sakurai, M. Izumi: This Bulletin, **1**, 297(1953).

The difference in the value of equivalent moles of Cl^- and H^+ liberation corresponds to the ring-formation, and H^+-liberation to the hydrolysis. Fig. 1 shows that the monofunctional ones are not subjected to hydrolysis within 10 hours under this condition in spite of their prompt ring-formation. It was already reported by Rydon[3] and Swain[3,4] that monofunctional nitrogen mustard, e.g. diethyl-β-chloroethylamine, forms a comparatively stable three-membered ring intermediate in aqueous solution, i.e. diethylethyleneimonium chloride, while the same ethyleneimonium compound transformed from the bifunctional one yields readily to hydrolysis. It is interesting that, in cases of N-oxides, the same relationship can be observed in the stability of the intermediates. This chemical property of the monofunctional compound may play a particular rôle in neurotropic action of these compounds, e.g. adrenergic blocking effect, but have no cancerocidal activities whether they be tertiary or N-oxide.

Concerning the velocity of ring-formation, however, it seems to depend thoroughly upon the property of each compound regardless of its functionality of the molecule. Out of the bifunctional compounds (Fig. 2), isoamyl-bis(β-chloroethyl)amine N-oxide was worth notice because it showed very prompt Cl^- liberation and practically no H^+ liberation within 10 hours. This compound compares favorably with HN_2 N-oxide in its cancerocidal activity and thus no definite relationship could be found between the velocity of Cl^- and H^+ liberation or between these chemical reactions and cancerocidal activity.

Tris(β-chloroethyl)amine N-oxide was characteristic in its comparatively prompt hydrolysis as shown in Fig. 3, and the slight fall of the Cl^- liberation curve after two hours meant the occurrence of secondary transformation to hydroxylamine derivative, by which reaction more Cl ions in the solution are rebound to the molecule than are liberated by hydrolysis.

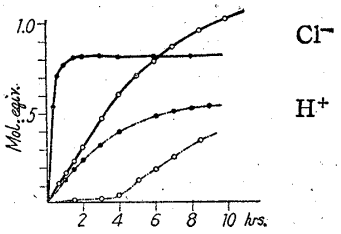


Fig. 3.
○: N,N′-Bis(β-chloroethyl)piperazine N,N′-dioxide.
•: Tris(β-chloroethyl) amine N-oxide
———: Cl^--liberation, —•—•—•—: H^+-liberation

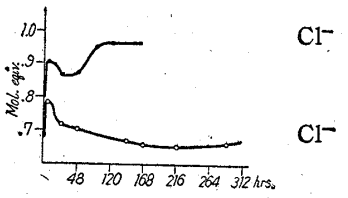


Fig. 4.
•: N-Benzyl-bis(β-chloroethyl) amine N-oxide
○: Dibenzyl-β-chloroethylamine N-oxide

Such decrease in liberated Cl^- at the last stage of reaction was observed in dibenamine N-oxide and benzyl-bis(β-chloroethyl)amine oxide (Fig. 4). The equilibrium of reaction inclined towards the formation of hydroxylamine derivative (c), as this product was sparingly

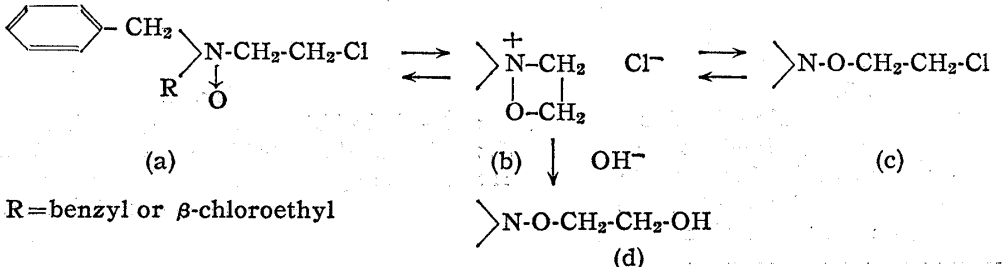


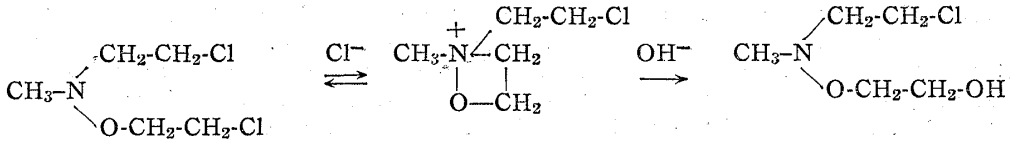
Fig. 5.

3) H. N. Rydon: J. Chem. Soc., 1947, 513.
4) C. G. Swain: J. Am. Chem. Soc., 69, 2971(1947).

soluble in water and separated from the reaction phase. The Cl^--liberation curve therefore declined notwithstanding the progress of hydrolysis.

The second ascent of the Cl^--liberation curve, observed in benzyl-bis(β-chloroethyl)-amine N-oxide and dibenamine N-oxide, can be understood as a gradual reverse of the reaction equilibrium from (c)→(b) in accordance with decrease in the concentration of (b) which occurs from hydrolysis of (b)→(d).

When a solution of methyl-bis(β-chloroethyl)amine N-oxide was kept for a period of time, the anions, i.e. OH^- and Cl^-, which existed in the solution, reacted competitively with the transformed dialkyl dimethylene-1,2-oximinium ion (I) (Fig. 6) and there resulted both N,N-methyl-β-chloroethyl-O-β-chloroethylhydroxylamine (II) and N,N-methyl-β-chloroethyl-O-β-hydroxyethylhydroxylamine (III) in a certain proportion.



(II) (I) (III)

Fig. 6.

As reaction (I)⇄(II) is reversible and (I) →(III) irreversible, the final product of the reaction mixture consisted of (III) alone.

However such a solution tended more or less to be subjected to intermolecular oxidation and to produce somewhat complicated products, because the N-oxide and especially its transformed product (I) were strongly oxidative substances. Such oxidation became more distinct when the medium was made strongly alkaline by addition of caustic alkali. The solution began to color within a few minutes and resulted in methyl-β-hydroxyethylamine in solution, in which reaction methyl-β-hydroxyethylcarbamic acid was assumed to be an intermediate. When solution of (I) was shaken with silver oxide to remove Cl^- completely or, enough amounts of sodium bicarbonate was added to maintain the solution slightly alkaline, the hydroxyl derivative (III) alone was formed in the solution.

If enough anion, such as Br^-, CH_3COO^-, or $C_6H_5COO^-$ was added to the solution of (I), the formation of bromoethyl, acetoxyethyl, or benzoxyethyl derivative of (III) was respectively confirmed.

Of these only the bromoethyl derivative was rather more susceptible to reverse reaction to (I) than the chloro derivative. Acetoxy or benzoxy derivative had no tendency to this reverse reaction.

The hydroxylamine derivatives were also isolated from tris(β-chloroethyl)amine N-oxide, benzyl-bis(β-chloroethyl)amine N-oxide, dibenzyl-β-chloroethylamine N-oxide, and diethyl-β-chloroethylamine N-oxide. Bergmann reported[5] that bis(β-chloroethyl)-β-hydroxyethylamine N-oxide was prepared by mere hydrolysis of tris(β-chloroethyl)amine N-oxide. It was described as an ether-soluble substance and its Reineckate to melt at 146°. However, the product which was obtained in this study employing the same proceduce as that of Bergmann's, was an oily substance and could be distilled without decomposition. It gave a picrylsulfonate, m.p. 127~129°, and a Reineckate of m.p. 144°, and was soluble in ether but insoluble in dilute hydrochloric acid. Catalytic reduction with PtO_2 resulted in bis(β-chloroethyl)amine as the chief product. Accordingly it seems correct to suppose that Bergmann's hydrolyzed product, as well as the present product, is N,N-bis(β-chloroethyl)-O-β-hydroxyethylhydroxylamine. N,N-Bis(β-chloroethyl)-O-β-chloroethylhydroxylamine was obtained as a by-product (picrylsulfonate, m.p. 144~145°), when tris(β-chloroethyl)amine was oxidized to N-oxide by the usual oxidizing method.[2]

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

The N-oxide, having no halogen, e.g. methyl-bis(β-acetoxyethyl)amine N-oxide, had no tendency to undergo this transformation reaction.

These transformations of the nitrogen mustard N-oxides are supposed to take place *in vivo* when they are injected into animals or humans and there might also be a possibility that anions of the cell constituents, e.g. carboxylic or phosphoric, are esterified in this mode of action, markedly different from that of the tertiary nitrogen mustards. These facts should be noted in studies of the cancerocidal action of the N-oxides

Experimental

Measurement of liberation of Cl^- and H^+—Titration was carried out by the method already reported.[1] The solutions of the compounds were all kept at 37°.

N,N-Benzyl-β-chloroethyl-O-β-chloroethylhydroxylamine (I) and N,N-Benzyl-β-chloroethyl-O-β-hydroxyethylhydroxylamine (II)—One g. of benzyl-bis(β-chloroethyl)amine N-oxide-HCl, dissolved in 20 cc. of water, was shaken with 2 g. of powdered Ag_2CO_3. After the filtrate was kept for 24 hrs. at 37°, the separated oil was extracted with ether. Ether was removed by evaporation and 1.5 g. of picrylsulfonic acid was added to the acetone solution of the residual oil. Acetone was then distilled off in vacuum and the residue rubbed well with a glass rod. It crystallized and after washing with ether, was recrystallized from butanol. The picrylsulfonate of (I) was a pale yellow, crystalline powder and melted at 108~109°; yield, 1 g. *Anal.* Calcd. for $C_{11}H_{15}ONCl \cdot C_6H_3O_9N_3S$: C, 37.71; H, 3.33; N, 10.35. Found: C, 37.77; H, 3.48; N, 10.38.

When the solution was kept at 37° for 72 hrs., the picrylsulfonate of (II) isolated by the same procedure. It was also a pale yellow crystalline powder and melted at 97~98°. *Anal.* Calcd. for $C_{11}H_{16}O_2NCl \cdot C_6H_3O_9N_3S$; C, 39.04; H, 3.63; N; 10.71. Found: C, 39.10; H, 3.68; N, 10.57.

N,N-Dibenzyl-O-β-hydroxyethylhydroxylamine—Obtained as picrylsulfonate by the same method from dibenzyl-β-chloroethylamine oxide-HCl. m.p. 166~166.5°. *Anal.* Calcd. for $C_{16}H_{19}O_2N \cdot C_6H_3O_9N_3S$: C, 48.00; H, 4,00. Found: C, 47.99; H, 3.95.

N,N-Dibenzyl-O-β-chloroethylhydroxylamine could not be isolated. These were very weak bases and their picrylsulfonates were readily dissociated to bases and acids in a solvent.

N,N-Methyl-β-chloroethyl-O-β-hydroxyethylhydroxylamine—Five g. of methyl-bis(β-chloroethyl)amine N-oxide-HCl (HN_2 N-oxide HCl) in 50 cc. of water was shaken with 7 g. of Ag_2O. After the filtrate was kept at 37° for 4 hrs., it was once more shaken with 7 g. of Ag_2O. The filtrate was extracted with ether and ether removed. The residue distilled in vacuum ($b.p_{10}$ 70~75°) without decomposition. Picrylsulfonate: Prisms, m.p. 147~148°, from acetone. *Anal.* Calcd. for $C_5H_{12}O_2NCl \cdot C_6H_3O_9N_3S$: C, 29.56; H, 3.35; N, 12.54. Found: C, 29.78; H, 3.37; N, 12.66.

This compound was obtanied by the following procedures: a) To a solution of the free base of HN_2 N-oxide was added a molar equivalent of $NaHCO_3$ and kept at 37° for several weeks. b) A solution of 2 g. of HN_2 N-oxide HCl in 20 cc. of $NaHCO_3$-saturated water was kept at 37° for 24 hrs. Yield, 0.5 g. as picrylsulfonate.

Change of HN_2 N-oxide HCl in strong alkaline solution—Two g. of HN_2 N-oxide HCl was dissolved in 20 cc. of 10% NaOH. The solution became strongly stained. After a few hours, the solution was extracted with ether and the ether solution was shaken with dilute HCl. To the acid layer, concentrated in vacuum, picrylsulfonic acid was added. The picrylsulfonate was recrystallized from a butanol-ethanol mixture. Pale yellow needles, m.p. 155~156°. *Anal.* Calcd. for $C_3H_9ON \cdot C_6H_3O_9N_3S$: C, 29.43; H, 3.72; N, 15.26. Found: C, 29.46; H, 3.64; N, 15.14.

N,N-Methyl-β-chloroethyl-O-β-bromoethylhydroxylamine—Two g. of HN_2 N-oxide-HCl, dissolved in 20 cc. of water, was shaken with 2.8 g. of powdered Ag_2CO_3. After 1 g. of $NaHCO_3$ and 6 g. of KBr were added to the filtrate, it was kept at a room temperature for 20 hrs. The separated oil was extracted with ether. The crude oil thus obtained was purified by distillation in vacuum ($b.p_2$ 69°). Picrylsulfonate: Prisms, m.p. 173~175°; yield, 2.7 g. *Anal.* Calcd. for $C_5H_{11}ONBrCl \cdot C_6H_3O_9N_3S$: C, 25.91; H, 2.75; N, 10.99. Found: C, 26.16; H, 2.81; N, 11.06.

N,N-Methyl-β-chloroethyl-O-β-acetoxyethylhydroxylamine (III) and N,N-Methyl-β-chloroethyl-O-β-benzoyloxyethylhydroxylamine (IV)—The procedures of preparation of these compounds were carried out in the same way as above. Equivalent amount of CH_3COONa or C_6H_5COONa was added to the reaction mixture instead of KBr. (III), yield, 3.1 g. from 5 g. of HN_2 N-oxide HCl, $b.p_3$ 95°. Picrylsulfonate, m.p. 115°. *Anal.* Calcd. for $C_7H_{14}O_3N$: C, 42.97; H, 7.16; N, 7.02. Found: C, 42.88; H, 7.10; N, 6.95.

As (III) was a very weak base, its picrate and picrylsulfonate were difficult to obtain analytically pure by recrystallization. Picrylsulfonate of (IV), m.p. 147~149°. *Anal.* Calcd. for $C_{12}H_{16}O_3N \cdot C_6H_3O_9N_3S$: C, 39.24; H, 3.45; N, 10.17. Found: C, 39.36; H, 3.64; N, 10.10.

Reverse reaction of N,N-methyl-O-β-chloro(bromo)ethylhydroxylamine to ring-oxide and HN_2 N-oxide—Two g. of the β-chloroethylhydroxylamine derivative was mixed with 20 cc. of 35%

HCl and boiled for 1 hour. After concentration in vacuum, water was added and then shaken with ether. Picric acid solution was added to the water layer. The separated picrate melted at 109~110° (from alcohol). No melting point depression was observed by admixture with the authentic sample of HN_2 N-oxide picrate.

One g. of the β-bromoethylhydroxylamine derivative was suspended in 10 cc. of water and shaken vigorously. Within 5 hrs., the oil was dissolved in water and picric acid solution was added immediately to the water layer. The separated picrate melted at 80~90°. It was recrystallized from petroleum ether and ethyl acetate and the pure picrate formed deep yellow needles, m.p. 93~95°. No melting point depression was observed by fusion with the authentic sample of N-β-chloroethyldimethylene-1,2-oximine N-methyl picrate.

N,N-Diethyl-O-β-hydroxyethylhydroxylamine—Eight g. of diethyl-β-chloroethylhydroxylamine N-oxide HCl was dissolved in water (100 cc.) and shaken with 16 g. of Ag_2CO_3. After the filtrate was kept at 37° for 24 hrs., 3.6 g $NaHCO_3$ was added and kept further at 37° for several weeks. When oxidative reaction of the solution ceased, the solution was acidified with HCl and evaporated to dryness in vacuum. The residue was extracted with dry acetone. The acetone was distilled off and the residue added to a solution of picrylsulfonic acid. The picrylsulfonate (plates) was separated (6 g.), and recrystallized from acetone, m.p. 148~149°. *Anal.* Calcd. for $C_6H_{15}ON \cdot C_6H_3O_9N_3S$: C, 33.80; H, 4.22; N, 13.15. Found: C, 33.96; H, 4.07; N, 13.08.

N,N-Diethyl-O-β-benzoyloxyethylhydroxylamine—Separated as oil from the solution of the free base of diethyl-β-chloroethylamine N-oxide when excess of C_6H_5COONa was added from the beginning into the reaction mixture. It formed a picrate, m.p. 106~107° (from alcohol) and a picrylsulfonate, m.p. 161~162°. *Anal.* Calcd. for $C_{13}H_{19}O_2N.C_6H_3O_9N_3S$: C, 50.67; H, 4.89; N, 12.44. Found: C, 50.45; H, 4.95; N, 12.29.

Catalytic reduction of Bergmann's hydrolyzed product of tris(β-choloethyl)amine N-oxide—Prepared acccording to Bergmann's description. Its picrylsulfonate melted at 127~129° and its Reineckate at 144°. *Anal.* Calcd. for $C_6H_{13}O_2NCl_2.C_6H_3O_9N_3S$: N, 11.31. Found: N, 11.45.

0.6 g. of this product, dissolved in 10 cc. of alcohol, was shaken with hydrogen and PtO_2 (100 mg.) at a room temperature. 70 cc. of hydrogen was absorbed (calcd. for 1 mol. equiv., 71 cc.), acidified with HCl, and evaporated to dryness. The residue formed a Reineckate (plates) of m.p. 153~154°, which showed no melting point depression with the authentic Reineckate of bis(β-chloroethyl)amine. *Anal.* Calcd. for $C_4H_9NCl_2 \cdot C_4H_7N_6CrS_4$: C, 20.87; H, 3.26. Found: C, 20.86; H, 3.33.

N,N-bis-β-Chloroethyl-O-β-chloroethylhydroxylamine—Obtained as a by-product by oxidizing tris(β-chloroethyl)amine as described in the previous paper.[1] Sparingly soluble in dil. HCl and soluble in ether, $b.p_5$ 117°. Picrylsulfonate, m.p. 144~145°, from a mixture of acetone and ether. *Anal.* Calcd. for $C_6H_{12}ONCl_3 \cdot C_6H_3O_9N_3S$: C, 28.04; H, 2.92; N, 10.91. Found: C, 28.17; H, 2.98; N, 10.88.

Methyl-bis(β-acetoxyethyl)amine oxide—Methyl-bis(β-acetoxyethyl)amine, $b.p_7$ 117~119°. Its picrylsulfonate melted at 109~111°. *Anal.* Calcd. for $C_9H_{17}O_4N \cdot C_6H_3O_9N_3S$: C, 36.25; H, 4.03; N, 11.29. Found: C, 36.45; H, 3.97; N, 11.24. 20 g. of the amine was dissolved in 22 g. of acetic anhydride and 200 cc. of 1*N* ethereal solution of H_2O_2. The reaction was exothermic and the mixture was warmed for 15 min. on a water bath after it cooled. The reaction mixture was shaken with water and the water layer evaporated in vacuum. A syrupy residue was obtained. It did not oxidize KI-starch reagent. Yield, 20~25 g. It gave a Reineckate of m.p. 122~123°, and a picrylsulfonate, m.p. 68~70° (from acetone and benzene). *Anal.* Calcd. for $C_9H_{17}O_5N \cdot C_4H_7N_6CrS_4$: C, 28.94; H, 4.46; N, 18.21. Found: C, 28.94; H, 4.31; N, 18.09. *Anal.* Calcd. for $C_9H_{17}O_5N \cdot C_6H_3O_9N_3S$: C, 35.15; H, 3.91. Found: C, 35.15: H, 3.65.

Change of Methyl-bis(β-acetoxyethyl)amine oxide—An aqueous solution of the free base of methyl-bis(β-acetoxyethyl)amine N-oxide, which was prepared by shaking the solution of its hydrochloride with Ag_2CO_3, did not submit to any transformation reaction when it was kept at 37° for 24 hrs. Even after 48 hrs., a picrylsulfonate of the unchanged N-oxide was obtained and no ether-soluble product was found. When the more concentrated solution of the free base (4.3 g. in 50 cc. H_2O) was warmed in vacuum until water was removed and then heated to 150° in an oil bath, also in vacuum (5 mm Hg), 2.5 g. of methyl-bis(β-acetoxyethyl)amine was obtained as a distillate.

Summary

The reaction of various kinds of tert-β-chloroethylamine N-oxides in aqueous solution was examined and it was found that the transformation reaction of HN_2 N-oxide, which had been reported previously, was a common reaction among the compounds of this type, although a marked difference in velocity of transformation was noticed.

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