A part of the expenses of this work was supported by the Grant-in-Aid for Scientific Research provided by the Ministry of Education to which the authors' thanks are due. Thanks are also due to Mr. D. Ohata, Iatrochemical Institute of Pharmacological Research Foundation, and to Misses M. Iwanaga and S. Ohno, Institute for Infectious Diseases, University of Tokyo, for the microanalyses.

Summary

9,10-Dihydro-9,10-phenanthrenediol cyclic phosphate (IV) was synthesized and its alcoholysis reaction with various hydroxylic compounds was investigated. The new cyclic phosphate was found to be alcoholyzed in acidic media by mono- and polyfunctional hydroxylic compounds, as well as by some aldoses, while the latter was found to be inert to the similar alcoholysis of hydrobenzoin or 4,4′-dinitrohydrobenzoin cyclic phosphates. D-Ribose 5-phosphate was produced by this type of reaction. The intermediate phosphodiester-type compound in the above alcoholysis reaction, alkyl 9,10-dihydro-9,10-phenanthrenediol phosphate, was found to be not stable enough in acid media to be detected by paper chromatography.

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99. Masahiro Torigoe : Studies on Carcinostatic Substances. XXXV.*1 Chemical and Antitumor Properties of Quaternary Derivatives of N-Alkoxy-2,2′-dichlorodiethylamine.

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

The previous investigation (Part XXXIV) revealed the strong antitumor activity of 2,2-bis(2-chloroethyl)isoxazolidinium chloride (I). One thing to be noted about this compound is that the compound (I) is regarded as a quaternary derivative of N,N,O-trisubstituted hydroxylamine. On the other hand, as reported in earlier stage of the investigation of this work,[1] N-(2-chloroethoxy)-N-methyl-2-chloroethylamine was neither effective on experimental tumor nor chemically active as an alkylating agent.

From these observations, attempt was made to prepare two new linear derivatives of (2-chloroethoxy)-bis(2-chloroethyl)methylammonium halide and some related compounds and discussions are made here on their chemical and biological properties. The compounds and their summarized properties to be discussed are shown in Table I.

The compounds (II), (III), (IV), and (V) were synthesized according to the processes shown in Chart 1.

As anticipated, (II) and (III) showed very potent antitumor activity against Yoshida sarcoma, while (IV) was completely inactive and (V) was slightly effective. Although even the latter two compounds yielded an active secondary amine, viz. 2,2′-dichlorodiethylamine, by catalytic reduction on more drastic condition than in case of reduction of (I), this reductive activation seemed not to occur *in vivo*.

Of course, (II) and (III) were proved to be reduced by milder reduction yielding N-

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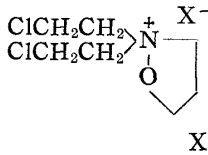
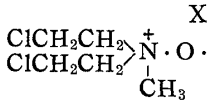
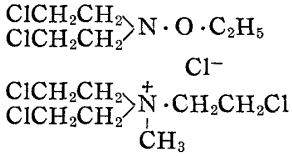
*1 This paper constitutes a part of a series entitled "Studies on Carcinostatic Substances" by M. Ishidate and Y. Sakurai. Part XXXIV : This Bulletin, **9**, 485 (1961).

*2 Designation now changed to Cancer Chemotherapy Section, Sasaki Institute, 26 Nishigahara 1-chome, Kita-ku, Tokyo (鳥越政宏).

1) M. Ishidate, *et al.* : Gann, **47**, 375 (1956).

Table I.

No.	Compound	$E\frac{1}{2}$ vs. S.C.E. pH 3.5	Thiosulfate consumption (mol. equiv.) 2 hr.	Thiosulfate consumption (mol. equiv.) 24 hr.	Cl^- liberation (mol. equiv.) 2 hr.	Cl^- liberation (mol. equiv.) 24 hr.	Toxicity on rat LD_{50} (mg./kg.)	Antitumor activity[a] against Yoshida sarcoma MTD (mg./kg.)	MED (mg./kg.)	MEC (mM)
(I)		-0.48_8		0.1～0.2		0.2 1.7 (8 days) 2.0 (15 days)	7.5	5	0.1	10^{-2}
(II)		-0.63_0	0.16	0.11	0.12	1.1_7	7.5	5	1	2.5×10^{-3}
(III)	*	-0.46_1	0.	0.5～0.7		1.4	7.5	5	0.5	5×10^{-4}
(IV)			0.	0.	0.	0.	175	100	—	
(VI)							75	50	—	

a) MTD : Maximum tolerance dose on rat (i.p.).
MED : Minimum effective dose on Yoshida sarcoma (i.p.).
MEC : Minimum effective concentration on Yoshida sarcoma cells *in vitro*.
— No effect

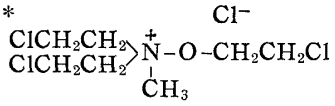
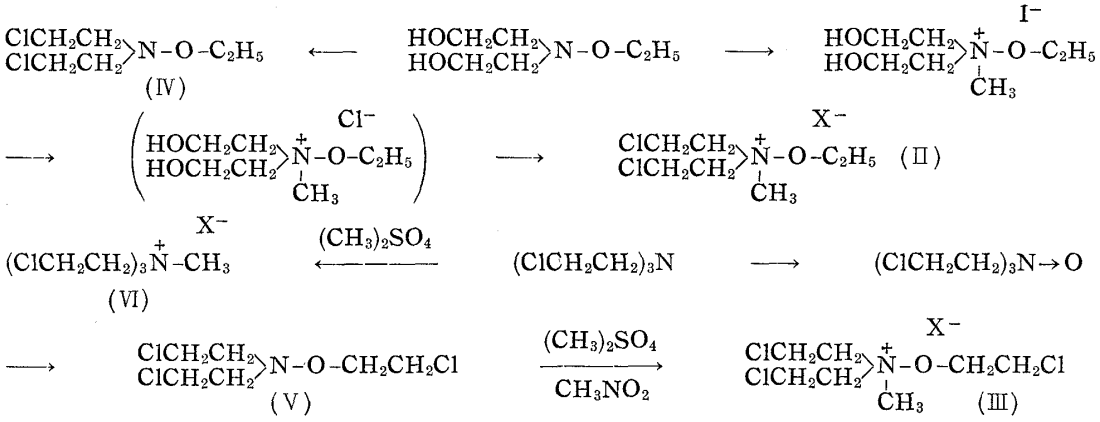
* 

Chart 1.

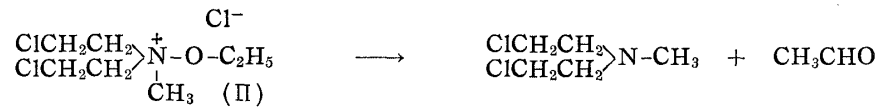


methyl-2,2′-dichlorodiethylamine and the presumption that they might be activated *in vivo* by the same mode of reduction as that of (I) would possibly be true.

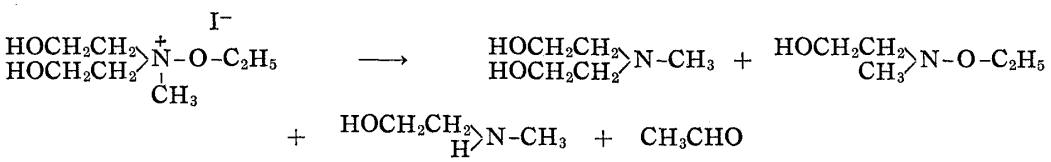
However, there were found some ambiguous points in understanding the chemical and biological attitude of (II) and (III). For instance, they are not so stable as expected against hydrolysis in an aqueous but non-reducing medium. As seen in Table I, velocity of Cl^- liberation based on hydrolysis of chlorine is far more rapid than that of (I) and such a high reactivity of chlorine, which is located at β-position of quaternary nitrogen, can hardly be anticipated, if the molecule is not submitted to reductive activation to the corresponding tertiary amine. The second question is independence on tumor cell population (tumor cell number/cc.) of values of minimum effective concentration (MEC) of these compounds against Yoshida sarcoma cultured *in vitro*, as reported by Imamura.[2] It seems to suggest that activation of this quaternary amine was not achieved by biological process but by pure chemical reactions.

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

To answer these questions, decomposition products of (II) and (III), produced by a long-term incubation at 37° in a neutral aqueous solution, were detected by paper chromatography. A spot developed by the Dragendorff reagent corresponding to the original substance faded and a few new spots appeared at smaller Rfs, which were almost identical with spots observed on chromatograms of the aged solution of N-methyl-2,2′-dichlorodiethylamine with the Dragendorff reagent. This preliminary result suggests that (II) and (III) yielded N-methyl-2,2′-dichlorodiethylamine simply when its neutral solution was kept at 37° without any reducing agent, and finally the decomposed products were actually isolated by the succeeding experiments. A solution of (II) was added to an extracting apparatus and extracted continuously with ether. A small amount of picric acid was dissolved in ether which was contained in a boiling flask of the apparatus. Tertiary amines and other ether-soluble products were gradually transferred to the ether extrct, from which amines were isolated as picrates. The decomposition process was therefore presumed as follows, although acetaldehyde was not isolated in this experiment :



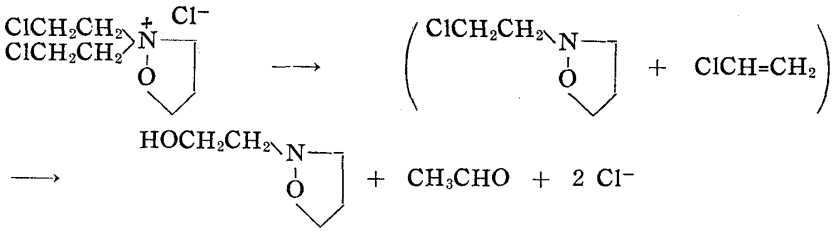
Ethoxybis(2-hydroxyethyl)methylammonium iodide was proved to be more resistant to decomposition than (II), but, when it was heated in a neutral aqueous solution at 100°, the following decomposed amines and the aldehyde as well were isolated :



From these results, it might be understood that this mode of decomposition can be regarded as a kind of Hofmann's exhaustive methylation, viz. β-dehydration of quaternary ammonium base, though it proceeds even in a dilute aqueous solution at room temperature. N-Methyl-2,2′-dichlorodiethylamine, liberated by this reaction might possibly play a chief rôle in exhibiting antitumor effect of the compounds. On the contrary, (VI) neither showed antitumor activity nor gave any decomposition products under the condition of incubation described above. In the past literature, derivatives of quaternary hydroxylamine, e.g. 1-methoxypyridinium iodide,[3] 1-methoxyquinolinium iodide,[4] methoxytrimethylammonium iodide,[5] and (2-hydroxyethyl)-methoxydimethylammonium iodide[6] were reported to decompose into the corresponding tertiary amines, liberating aldehydes, but the reaction conditions were generally more drastic than in the case of (I) and (II).

The experiment was extended to the study of decomposition of (I). As shown in Table I, (I) liberates almost 2 molar equivalents of Cl^- after incubation for 10 days or more in a neutral aqueous solution. This aged solution gave no spot by the Dragendorff reagent on its paper chromatogram. During the period of hydrolysis, a volatile product was caught in a solution of dimedone, and was identified as acetaldehyde as an addition product with dimedone. From the aqueous solution, 2-isoxazolidineëthanol was isolated and identified as 3,5-dinitrobenzoate. The reaction is therefore assumed as follows, which is deemed also as similar β-dehydration reaction :

3) E. Ochiai : J. Org. Chem., **18**, 546 (1953).
4) M. Henze : Ber., **70**, 1270 (1937).
5) J. Meisenheimer : Ann., **397**, 273 (1913).
6) L.W. Jones, R.T. Major : J. Am. Chem. Soc., **49**, 1527 (1927).



On the contrary, a solution of 2,2-diethylisoxazolidinium chloride, which has no chlorine on the side chains, does not change even on heating at 100° for 1 hour.

In conclusion, quaternary derivatives of N-alkoxy-2,2′-dichlorodiethylamine are regarded as a kind of masked compounds and activated *in vivo* in two ways; the one by biological reduction and the other by pure chemical β-dehydration reaction. The former reaction seemed to be predominant in the case of isoxazolidinium derivatives, but in the case of linear derivatives such as (II) and (III), the latter reaction is assumed to be the chief one. Reduction potentials by polarography[7] of (II) and (III) were not so different from (I), as demonstrated in Table I.

Experimental

O-Ethylacetoxime (VII)——Acetoxime (278 g.) dissolved in water (278 cc.) was added with 5*N* NaOH (835 cc.) at 16～18°. Into this solution, 455 g. of EtBr was added dropwise at 32～40° with vigorous stirring. The mixture was then heated with continuous stirring until the temperature of the vapor reached 67.5°. The mixture was then chilled to 2° and the oil that separated was dried over $CaCl_2$. Yield, 218 g., b.p. 90～93.5°.

Ethoxyamine (VIII)——A mixture of (VII) (218 g.), 17% HCl (1180 cc.), and hydroquinone (2～3 mg.) was heated at 80～90° for 1.5 hr. Evaporation of the mixture in a reduced pressure afforded a crystalline residue (103 g.), which was added with powdered NaOH (176 g.) and the mixture was fractionated as a whole. Yield, 49.5 g., b.p. 68～69°.

N-Ethoxy-2,2′-dihydroxydiethylamine (IX)——Through a mixture of (VIII) (35 g.) and water (153 cc.), ethylene oxide (prepared from 174 g. of ethylenechlorohydrin and 720 cc. of 5*N* NaOH) was passed at 5°±1°. After being kept at 8～10° for 2 or 3 days, water was removed *in vacuo* and the residue was distilled *in vacuo* to give 67 g. of (IX), $b.p_{1.5}$ 110～115°. *Anal.* Calcd. for $C_6H_{15}O_3N$: C, 48.33; H, 10.14; N, 9.39. Found : C, 48.17; H, 10.07; N, 9.24.

Its picrate was recrystallized from AcOEt-benzene mixture and exsiccated *in vacuo* over paraffin. m.p. 92～94°. *Anal.* Calcd. for $C_{12}H_{18}O_{10}N_4$: C, 38.10; H, 4.79; N, 14.81. Found : C, 38.29; H, 4.74; N, 14.65.

N-Ethoxy-2,2′-dichlorodiethylamine (X)——Synthesis of (X) was carried out after the method of preparation of N-methoxy-2,2′-dichlorodiethylamine by Jones, *et al.*[8] $b.p_{15}$ 98～102°. *Anal.* Calcd. for $C_6H_{13}ONCl_2$: C, 38.72; H, 7.04; N, 7.53. Found : C, 38.91; H, 7.03; N, 7.61.

Ethoxy-bis(2-hydroxyethyl)methylammonium Iodide (XI)——A mixture of (IX) (59 g.), MeI (30.4 cc.), and MeOH (10 cc.) was heated in a sealed tube at 70° for 1～2 hr. After removal of volatile components, the residue was recrystallized from Me_2CO. m.p. 70～73°. Yield, 70.6 g. *Anal.* Calcd. for $C_7H_{18}O_3NI$: C, 28.88; H, 6.23; N, 4.81. Found : C, 28.85; H, 6.00; N, 4.84.

Bis(2-chloroethyl)ethoxymethylammonium Iodide (II)——A solution of (XI) (44 g.) in water (150 cc.) was agitated with AgCl (freshly prepared from 48 g. of $AgNO_3$) for 30 min. Removal of AgI by filtration and distillation of water below 50° gave a colorless syrupy residue, which was mixed with $SOCl_2$ (150 g.) at 5～15°. After standing overnight at room temperature, excess of $SOCl_2$ was evaporated *in vacuo*. The crude chloride (11.8 g.) thus obtained was dissolved in water (4 cc.) and extracted once with Et_2O to remove impurities. The solution was added with 50% KI (16.6 g.) and a crystalline precipitate was immediately produced. m.p. 93° (from MeOH or EtOH). *Anal.* Calcd. for $C_7H_{16}ONCl_2I$: C, 25.63; H, 4.93; N, 4.27. Found : C, 25.52; H, 4.70; N, 4.26.

A mixture of the crude chloride (5 g.), water (5 cc.), and excess of $NaHCO_3$ was extracted repeatedly with Et_2O, and the water layer was added with ethanolic solution of picric acid. m.p. 107～108° (from MeOH). Yield, 3.7 g. *Anal.* Calcd. for $C_{13}H_{18}O_8N_4Cl_2$: C, 36.38; H, 4.23; N, 13.05. Found: C, 36.24; H, 4.17; N, 12.91.

7) I. Aiko : This Bulletin, **1**, 335 (1953).
8) E.H. Jones, W. Wilson : J. Chem. Soc., **1949**, 547.

N-(2-Chloroethoxy)-2,2′-dichlorodiethylamine (V)——A mixture of 2,2′,2″-trichlorotriethylamine N-oxide hydrochloride (7.7 g.), water (60 cc.), KCl (11.4 g.), and $NaHCO_3$ (5.5 g.) was incubated at 25～30° for 20 hr. The oil that separated was taken up in Et_2O which was washed first with 2% HCl and finally with water. The Et_2O solution was dried over anhyd. Na_2SO_4 and fractionated *in vacuo*. b.p_1 106～107°. Yield, 4.5 g. *Anal.* Calcd. for $C_6H_{12}ONCl_3$: C, 32.68; H, 5.48; N, 6.35. Found: C, 32.43; H, 5.21; N, 6.13.

(2-Chloroethoxy)-bis(2-chloroethyl)methylammonium Chloride (III)——A mixture of (V) (15 g.), Me_2SO_4 (13.6 cc.), and $MeNO_2$ (13.6 cc.) was heated for 45 min. in an oil bath kept at 100°. After cool, the mixture was added with Et_2O (240 cc.) and the oil, insoluble in Et_2O, was washed repeatedly with Et_2O. After washing, the oil was dissolved in water (30 cc.), neutralized with $NaHCO_3$, and the mixture was washed with Et_2O again. From the aqueous solution, the methopicrate was precipitated by addition of aqueous solution of sodium picrate. Yield, 4 g., m.p. 91～91.5° (from EtOH). *Anal.* Calcd. for $C_{13}H_{17}O_8N_4Cl_3$: C, 33.67; H, 3.69; N, 12.08; Cl, 22.94. Found: C, 33.50; H, 3.45; N, 11.84; Cl, 22.96.

The above picrate was converted to the chloride in the usual manner. m.p. 99° (from dehyd. EtOH). *Anal.* Calcd. for $C_7H_{15}ONCl_4$: C, 31.02; H, 5.58; N, 5.17. Found: C, 30.95; H, 5.36; N, 5.13.

Tris(2-chloroethyl)methylammonium Chloride (VI)——A mixture of triethanolamine (10 g.), MeI (20 g.), and EtOH (10 cc.) was refluxed for 2 hr. The cooled solution was filtered from a small amount of insoluble impurites and the solvent and excess of MeI were evaporated *in vacuo*. The residue (18 g.) was washed five times with Me_2CO (10 cc. each) and dissolved in water (62 cc.). The solution was agitated with AgCl (freshly prepared from 19.6 g. of $AgNO_3$) for 30 min. Filtration of AgI and distillation by *vacuo* gave a syrupy residue (9.3 g.), 2.9 g. of which was heated with $SOCl_2$ (25.8 g.) at 70～80° for 2 hr. After removal of $SOCl_2$, the crystalline residue was recrystallized from BuOH. m.p. 198～199° (decomp.). Yield, 1.3 g. *Anal.* Calcd. for $C_7H_{15}NCl_4$: C, 32.97; H, 5.93; N, 5.49. Found: C, 32.87; H, 5.95; N, 5.48.

Picrate: m.p. 111°. *Anal.* Calcd. for $C_{13}H_{17}O_7N_4Cl_3$: C, 34.87; H, 3.83; N, 12.52. Found: C, 34.75; H, 3.73; N, 12.58.

Decomposition of (II)——A mixture of the chloride of the subject compound, obtained from its pure picrate (0.86 g.), and water (0.23 cc.) was kept for 100 hr. at room temperature for continuous extraction using the automatic extraction apparatus. A small amount of picric acid was added previously to Et_2O in the boiling flask of the apparatus. During the extraction, a yellow picrate began to precipitate out gradually (0.175 g. in total). m.p. 129～131° (from MeOH). It showed no melting point depression on admixture with the authentic specimen of N-methyl-2,2′-dichlorodiethylamine picrate. *Anal.* Calcd. for $C_{11}H_{14}O_7N_4Cl_2$: C, 34.30; H, 3.66; N, 14.55. Found: C, 34.30; H, 3.36; N, 14.88.

Decomposition Reaction of (XI)——A mixture of (XI) (1.45 g.), $NaHCO_3$ (1.68 g.), and water (5 cc.) was heated at 90～100° for 1 hr. In the course of heating, liberation of acetaldehyde was detected by leading it into 1 cc. of 10% ethanolic dimedone solution, which was previously chilled to −20°. Colorless crystals, m.p. 138.5～140°, separated from the dimedone solution and were identified with the authentic specimen of acetaldehyde-dimedone adduct by mixed m.p. determination. *Anal.* Calcd. for $C_{18}H_{26}O_4$: C, 70.56; H, 8.55. Found: C, 70.48; H, 8.39.

The reaction mixture, separated from the volatile fraction, was extracted with $CHCl_3$. The extract was concentrated to a small volume and added with Et_2O solution of picric acid. The precipitate (0.47 g.) was collected and recrystallized from water using activated charcoal. m.p. 137～139°. It was identified by analysis with the picrate of N-ethoxy-N-methyl-2-hydroxyethylamine. *Anal.* Calcd. for $C_{11}H_{16}O_9N_4$: C, 37.93; H, 4.63; N, 16.09. Found: C, 37.82; H, 4.02; N, 15.99.

The aqueous solution left after extraction with $CHCl_3$ was acidified with HCl and evaporated to dryness in a reduced pressure. After addition of 50% NaOH (1 cc.), the free amine was extracted with $CHCl_3$ and precipitated as its picrate (0.43 g.) (from water), m.p. 144.5～146.5°, undepressed on admixture with the authentic picrate of 2-methylaminoethanol. *Anal.* Calcd. for $C_9H_{12}O_8N_4$: C, 35.59; H, 3.67; N, 18.58. Found: C, 35.53; H, 3.98; N, 18.42.

The mother liquor of the above picrate (m.p. 144.5～146.5°) was evaporated *in vacuo* and the residue (0.16 g.) was recrystallized from water, m.p. 89～90°, undepressed on admixture with the authentic specimen of N-methyl-2,2′-dihydroxydiethylamine picrate. *Anal.* Calcd. for $C_{11}H_{16}O_9N_4$: C, 37.93; H, 4.63; N, 16.09. Found: C, 37.86; H, 4.44; N, 16.11.

Catalytic Reduction of (II)——(II) (0.24 g.), obtained from its pure picrate, was dissolved in H_2O (1 cc.) and shaken with H_2 at room temp. over Pd-C (prepared from 0.1 g. of activated charcoal and 2 cc. of 0.5% $PdCl_2$). Within 1 hr., 20 cc. of H_2 was absorbed (1 molar equivalent: 22.4 cc.). The filtrate from the reaction mixture was added with sodium picrate and the picrate (0.25 g.), m.p. 127～129°, precipitated immediately. It was identified with N-methyl-2,2′-dichlorodiethylamine picrate by mixed m.p. determination with the authentic sample. *Anal.* Calcd. for $C_{11}H_{14}O_7N_4Cl_2$: C, 34.30; H, 3.66; N, 14.55. Found: C, 34.49; H, 3.51; N, 14.42.

Catalytic Reduction of (III)——(III) (0.27 g.) was dissolved in H_2O (3.3 cc.) and shaken with H_2 at room temperature over Pd-C (prepared from 5 mg. of activated charcoal and 1 cc. of 0.5% $PdCl_2$). Within 1 hr., 22 cc. of H_2 was absorbed. The filtrate from the reaction mixture was added with 0.2*N* sodium picrate (3 cc.) and the picrate that precipitated (0.23 g.) melted at 131~132°. It was identified with N-methyl-2,2'-dichlorodiethylamine picrate by mixed m.p. determination with the authentic specimen. *Anal.* Calcd. for $C_{11}H_{14}O_7N_4Cl_2$: C, 34.30; H, 3.66; N, 14.55. Found : C, 34.15; H, 3.53; N, 14.65.

Catalytic Reduction of (XI)——(XI) (0.58 g.) was dissolved in water (1 cc.) and agitated with AgCl (freshly prepared from 0.8 g. of $AgNO_3$) for 30 min. After filtration and washing of the residue with hot water (2.5 cc.), the filtrate and washings were combined and shaken with H_2 at room temp. over Pd-C (prepared from 0.2 g. of activated charcoal and 4 cc. of 0.5% $PdCl_2$). Within 30 min., 42 cc. of H_2 was absorbed (1 molar equivalent : 44.8 cc.). The filtrate from the reaction mixture was evaporated to dryness in a reduced pressure, the residue was basified with 50% NaOH (0.5 cc.), and extracted with $CHCl_3$, which was concentrated to a small volume and added with an ethereal solution of picric acid.

Picrate : m.p. 92.5~93.5°(from water) (0.5 g.). It showed no m.p. depression with the authentic specimen of N-methyl-2,2'-dihydroxydiethylamine picrate. *Anal.* Calcd. for $C_{11}H_{16}O_9N_4$: C, 37.93; H, 4.63; N, 16.09. Found : C, 38.20; H, 4.41; N, 16.10.

Decomposition of (I)——A mixture of (I) (0.4 g.), $NaHCO_3$ (0.67 g.), and water (3 cc.) was incubated at 37° for 15 days. In the course of this decomposition reaction, a volatile substance liberated, which was captured in an aqueous 0.23% dimedone solution (170 cc.). An addition product separated gradually in the course of incubation. Yield, 0.12 g., m.p. 139~140.5°(from EtOH), undepressed on admixture with the authentic specimen of acetaldehyde-dimedone adduct. *Anal.* Calcd. for $C_{18}H_{26}O_4$: C, 70.56; H, 8.55. Found : C, 70.45; H, 8.62.

The above reaction mixture separated from the volatile fraction was extracted with Et_2O and another reaction product was extracted thoroughly 15 times with 2 cc. each of $CHCl_3$. After removal of the solvent, the residue (0.16 g.) was dissolved in pyridine (0.8 cc.) and added with powdered 3,5-dinitrobenzoyl chloride (0.32 g.) with ice cooling. After being kept in a refrigerator overnight, the mixture was diluted with water (2 cc.), extracted with AcOEt, and washed with dilute solution of $NaHCO_3$. Evaporation of the solvent gave a crystalline residue (0.33 g.), m.p. 106°(from EtOH). *Anal.* Calcd. for $C_{12}H_{13}O_7N_3$: C, 46.30; H, 4.21; N, 13.50. Found : C, 46.21; H, 3.83; N, 13.11.

Determination of Cl^- Liberation and Thiosulfate Consumption in $NaHCO_3$-buffered Solution——Titration was carried out by the procedure completely analogous to those described in the preceding report.[9]

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Summary

Alkoxy-bis(2-chloroethyl)methylammonium halides were prepared and their chemical reactivity and latent antitumor activity against the Yoshida sarcoma were examined. Chemical mechanism of activation of these compounds was also discussed.

(Received October 27, 1960)

9) M. Ishidate, *et al.* : This Bulletin, **6**, 164 (1958).