UDC 615.771.7:547.233'222

108. **Morizo Ishidate, Yoshio Sakurai, and Masahiro Torigoe** : Studies on Carcinostatic Substances. XXXVI.[*1] A New Type of Antitumor Derivatives of Nitrogen Mustard with Latent Activity.

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

It was reported in the preceding paper of this series, that N-alkoxy-2,2'-dichlorodiethylamine methochloride is a kind of derivatives of nitrogen mustard with latent activity because it transforms into the active form *in vivo*, viz. N-methyl-2,2'-dichlorodiethylamine, by β-dehydration process. Contrary to such a quaternary ammonium form, N-alkoxy-2,2'-dichlorodiethylamine remains inactive even after a long-term incubation *in vitro* or when administered to tumor animals.

Recently, it was noticed, however, that one of such tertiary hydroxy-amine derivatives, N-(2-chloroethoxy)-2,2'-dichlorodiethylamine (IV) exhibits a weak antitumor activity against Yoshida sarcoma either *in vitro* or *in vivo* and this observation led to the preparation of a series of derivatives of N-(2-substituted ethoxy)-2,2'-dichlorodiethylamine. The compounds discussed in this paper are shown in Table I.

Table. I.

No.	Compound $\begin{matrix}ClCH_2CH_2\\ClCH_2CH_2\end{matrix}{>}N\text{-}O\text{-}CH_2CH_2\text{-}X$	Thiosulfate consumption (mol. equiv.)		Cl$^-$ liberation (mol. equiv.)		Toxicity on rat LD$_{50}$	Antitumor activity[a] against Yoshida sarcoma		
	X	2 hr.	24 hr.	2 hr.	24 hr.	(mg./kg.)	MTD (mg./kg.)	MED (mg./kg.)	MEC (m*M*.)
(I)	-OH								—
(II)	-H	0	0	0	0	175	100	—	
(III)	-OC$_2$H$_5$								—
(IV)	-Cl	0	0.29	0.04	0.27	75	50	10	10^{-2}
(V)	-Br	0.54	1.4	0.56	1.34	75	50	1	10^{-3}
(VI)	-O-SO$_2$-CH$_3$	0.12	1.0	0.1	0.65	75	50	10	10^{-3}
(VII)	-O-SO$_2$-⬡-CH$_3$	0.09	0.63	0.02	0.55	37.5	25	2.5	10^{-2}
(VIII)	-O-CO-⬡-SO$_3$H·C$_5$H$_5$N	0	0	0	0	1000	250	—	
(IX)	-O-CO-CH$_3$								—
(X)	-O-CO-⬡								—

a) MTD : Maximum tolerance dose on rat
MED : Minimum effective dose on Yoshida sarcoma rat
MEC : Minimum effective concentration on *in vitro*-cultured Yoshida sarcoma cells
— : No effect

The preparation of (I), (II), and (IV) was already described in the preceding report[1] but the preparation procedure of (I) was improved in the present work. (V) was easily obtained by the method similar to the case of (IV). Attempt to obtain (VI) and (VII) by the reaction of 2,2',2''-trichlorotriethylamine N-oxide and sodium sulfonate in a neutral aqueous medium was not successful and digestion of ethanolic mixture of (V) and silver *p*-toluenesulfonate yielded (III) alone. Preparation of the two sulfonic esters (VI) and (VII) succeeded only by the reaction of (I) with sulfonyl chloride in pyridine. *p*-Chlorosulfonylbenzoic acid was treated with (I) by the same procedure as that for (VI) in order to obtain N-(2-*p*-carboxybenzenesulfonyloxyethoxy)-2,2'-dichlorodiethylamine, but the only product

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[*1] Part XXXV : This Bulletin, **9**, 613 (1961).

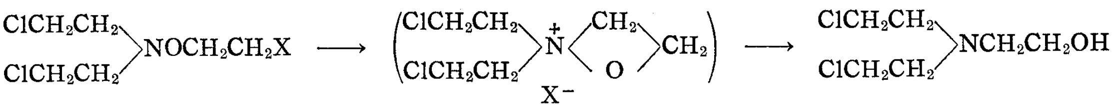
[*2] Designation now changed to Cancer Chemotherapy Section, Sasaki Institute. 26 Nishigahara 1-chome, Kita-ku, Tokyo (石舘守三, 桜井欽夫, 鳥越政宏).

1) S. Owari : This Bulletin, **1**, 353 (1953); M. Torigoe : *Ibid.*, **9**, 613 (1961).

of this reaction was nothing other than (Ⅷ), which has no antitumor activity. N-(2-Acetoxyethoxy)-2,2′-dichlorodiethylamine and N-(2-benzoyloxyethoxy)-2,2′-dichlorodiethylamine were also prepared and proved to have no antitumor activity.

It was suggested by the past experiments that the activation of 2,2-bis(2-chloroethyl)-isoxazolidinium chloride or even N-alkoxy-2,2′-dichlorodiethylamine methochloride is due to the reductive cleavage of N-O bond, but it seems to be difficult to assume that such reduction takes place at N-O bond of these tertiary hydroxylamine derivatives.

In fact the N-O bond resisted catalytic reduction in acid medium, in which the corresponding quaternary derivatives could be reduced. However, it was found that (V) slowly absorbed about 0.7 molar equivalent of hydrogen in a medium of pH 7 during 6 hours and unexpectedly yielded N-(2-hydroxyethyl)-2,2′-dichlorodiethylamine,[2] which was detected by paper chromatography. This finding suggests a scheme of its reaction in a neutral solution as follows:



If the two sulfonic esters (VI and Ⅶ) do not change to such an intermediate ring structure, the antitumor effect can never be expected because of their monofunctional alkylating reactivity of their single sulfonic ester group. This process shows the activating reaction in reducing environment or *in vivo*. It was regarded however that the intermediate, 2,2-bis(2-chloroethyl)-1,2-oxazetidinium chloride,[3] does not accumulate in a neutral aqueous solution of (V) incubated at 37° for a long period, because it could not be detected by polarographic determination[3] of the reaction mixture all during the experiment.

From the view of chemotherapeutic index (LD_{50}/minimum effective dose) against Yoshida sarcoma rat, (V) is comparable to N-methyl-2,2′-dichlorodiethylamine N-oxide, but the insoluble nature of this compound hinders its practical application. However, it may be said that this kind of a compound should be regarded as a new type of derivative with latent activity and is worth being further investigated.

Experimental

N-(2-Hydroxyethoxy)-2,2′-dichlorodiethylamine (I)——Into a suspension of Ag_2CO_3 (prepared from 120 g. of $AgNO_3$) in H_2O (220 cc.), an aqueous solution (180 cc.) of 2,2′,2″-trichlorotriethylamine N-oxide hydrochloride (40 g.) was poured slowly with vigorous stirring. During the reaction, solid $NaHCO_3$ (40 g.) was added in small portions to the reaction mixture so as to maintain its pH constant at about 9. Stirring was continued until the mixture lost its oxdizing property against KI solution. After extraction with AcOEt (480 cc.), the extract was distilled off *in vacuo*. The oily residue (17.5 g.) was dissolved in benzene-petr. ether (3:4) mixture (34 cc.) and poured on an Al_2O_3 column (175 g.), which was washed first with the same solvent and secondarily with benzene. Finally (I) was eluted by EtOH. Yield, 14 g. Its picrylsulfonate melted at 127° which was proved identical with that described in the preceding report.[1]

N-(2-Ethoxyethoxy)-2,2′-dichlorodiethylamine (III)——A mixture of silver *p*-toluenesulfonate (0.68 g., well dried and powdered), (V) (0.27 g.), and dehyd. EtOH (3 cc.) was refluxed for 1 hr. and the solvent was removed *in vacuo*. The reaction product was extracted with Et_2O and the residue of the Et_2O extract (0.08 g.) was converted to its picrylsulfonate. Fine colorless needles, m.p. 113~114° (from Me_2CO-petr. ether). Yield, 0.045 g. *Anal.* Calcd. for $C_{14}H_{20}O_{11}N_4Cl_2S$: C, 32.13; H, 3.85; N, 10.71. Found: C, 32.13; H, 3.71; N, 10.68.

N-(2-Bromoethoxy)-2,2′-dichlorodiethylamine (V)——A solution of 2,2′,2″-trichlorotriethylamine N-oxide hydrochloride (5.2 g.) in H_2O (40 cc.) was agitated with Ag_2CO_3 (freshly prepared from 6.8 g. of $AgNO_3$) at 0°. After filtration, the filtrate was added with KBr (29.8 g.) and incubated at 37° for

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[2] S. Owari: This Bulletin, **3**, 345 (1955).
[3] I. Aiko: *Ibid.*, **1**, 335 (1953).

1 hr., during which the mixture was kept at pH 4 by addition of 47% HBr or $NaHCO_3$. Then it was added with an excess of $NaHCO_3$ and the oil that separated was taken up in Et_2O (60 cc.). The combined Et_2O extract was washed with 2% HCl (80 cc.) and then H_2O, the Et_2O layer was dried over anhyd. Na_2SO_4, and fractionated *in vacuo*, b.p$_3$ 123~123.8°. Yield, 3.5 g. *Anal.* Calcd. for $C_6H_{12}ONBrCl_2$: C, 27.19; H, 4.56; N, 5.29. Found : C, 27.06; H, 4.42; N, 5.39.

Picrylsulfonate : m.p. 138~139°(from Me_2CO-Et_2O). *Anal.* Calcd. for $C_{12}H_{15}O_{10}N_4BrCl_2S$: C, 25.82; H, 2.70; N, 10.04. Found : C, 25.92; H, 2.78; N, 10.05.

N-(2-Acetoxyethoxy)-2,2′-dichlorodiethylamine (IX)——A solution of 2,2′,2″-trichlorotriethylamine N-oxide hydrochloride (2.6 g.) in H_2O (20 cc.) was agitated with Ag_2CO_3 (freshly prepared from 3.4 g. of $AgNO_3$). After filtration, the filtrate was added with $NaHCO_3$ (1 g.) and AcONa (3.3 g.), and incubated at 25~30° for 20 hr. The product was extracted and purified by the same procedure as in the case of (V); b.p$_3$ 151~154°. *Anal.* Calcd. for $C_8H_{15}O_3NCl_2$: C, 39.36; H, 6.19; N, 5.74. Found: C, 39.22; H, 6.26; N, 5.77.

N-(2-Benzoyloxyethoxy)-2,2′-dichlorodiethylamine (X)——This was prepared by a similar procedure as that for (V), b.p$_{0.09}$ 165°. *Anal.* Calcd. for $C_{13}H_{17}O_3NCl_2$: C, 50.99; H, 5.60; N, 4.58. Found: C, 50.83; H, 5.59; N, 4.61.

N-(2-*p*-Toluenesulfonyloxyethoxy)-2,2′-dichlorodiethylamine (VII)——(I) (0.51 g.) was tosylated with *p*-toluenesulfonyl chloride (0.53 g.) in pyridine (0.8 cc.) with chilling to −5° with ice-salt mixture. After being placed in a refrigerator of 8° overnight, the mixture was added with ice (2 g.) and extracted with Et_2O. The extract was washed with 2% HCl, dried, and evaporated. The oily residue that remained (0.69 g.) was dissolved in benzene-petr. ether (3:4) mixture (7 cc.) and poured on an column of Al_2O_3 (5 g.). The column was eluted by the same solvent and the eluate was evaporated to leave a colorless syrupy residue. Yield, 0.59 g. *Anal.* Calcd. for $C_{13}H_{19}O_4NCl_2S$: C, 43.82; H, 5.37; N, 3.93. Found : C, 43.84; H, 5.28; N, 4.14.

Picrylsulfonate : Colorless needles, m.p. 140~141°(from Me_2CO-Et_2O). *Anal.* Calcd. for $C_{19}H_{22}O_{13}N_4Cl_2S_2$: C, 35.14; H, 3.41; N, 8.63. Found : C, 35.21; H, 3.17; N, 8.80.

N-(2-Methanesulfonyloxyethoxy)-2,2′-dichlorodiethylamine (VI)——This was prepared from (I) (0.51 g.) by the same procedure as in the case of (VII). Colorless syrup, 0.16 g. *Anal.* Calcd. for $C_7H_{15}O_4NCl_2S$: C, 30.00; H, 5.39; N, 5.00. Found : C, 29.70; H, 5.36; N, 5.09.

Picrylsulfonate : Colorless needles, m.p. 128~129°(from Me_2CO-Et_2O). *Anal.* Calcd. for $C_{13}H_{18}O_{13}N_4Cl_2S_2$: C, 27.23; H, 3.16; N, 9.77. Found : C, 27.29; H, 3.06; N, 9.76.

N-[2-(*p*-Carboxybenzenesulfonyloxy)ethoxy]-2,2′-dichlorodiethylamine (VIII)——(I) (4 g.) was treated with *p*-chlorosulfonylbenzoic acid[4] (4.9 g.) by the same procedure as in the case of (VII). The reaction mixture was diluted with H_2O (16 cc.) and the unreacted acid was extracted with Et_2O. After acidification to Congo Red, the product was extracted with $CHCl_3$. On evaporation of the solvent, the extract gave a crystalline residue (6 g.) of pyridine salt of (VIII) as colorless prisms, m.p. 100~102° (from dehyd. EtOH-Et_2O). *Anal.* Calcd. for $C_{18}H_{22}O_6N_2Cl_2S \cdot \frac{1}{2}H_2O$: C, 45.58; H, 4.89; N, 5.91. Found: C, 45.41; H, 4.82; N, 5.99.

By drying at 60° for 1 hr., the anhydrous salt was obtained. *Anal.* Calcd. for $C_{18}H_{22}O_6N_2Cl_2S$: C, 46.46; H, 4.76. Found : C, 46.37; H, 4.88.

A picrate precipitated from its aqueous solution by addition of aqueous picric acid as yellow needles, m.p. 165~166°, which was identical with the authentic specimen of pyridine picrate.

pH value of 0.1*N* aqueous solution of the above pyridine salt (VIII) was as small as 3.13, while those of benzoic acid-pyridine salt and pyridine hydrochloride were found to be 4.65 and 3.08, respectively. The free acid form of (VIII) was not soluble in Et_2O.

Catalytic Reduction of a Neutral Solution of (V)——A mixture of activated charcoal (0.1 g.) and 1% $PdCl_2$ (1 cc.) was shaken with H_2. Into this catalyst suspension, KH_2PO_4 (0.14 g.), $Na_2HPO_4 \cdot 2H_2O$ (0.18 g.), (V) (0.26 g.), and EtOH (5 cc.) were added and shaken again with H_2. Within 6 hr. about 15 cc. of H_2 was absorbed. The catalyst was removed by filtration, the filtrate (pH 6~7) was acidified with HCl, concentrated to a small volume in a reduced pressure, and extracted with Et_2O. From the Et_2O extract, 0.05 g. of the starting material was recovered. The aqueous layer was evaporated to dryness and extracted with hot Me_2CO (6 cc.). The paper chromatogram of the Me_2CO extract (syrup, 0.04 g.) gave two spots of Rf 0.12 and of 0.45 (Dragendorff reagent). Rf of the authentic specimen of N-2-hydroxyethyl-2,2′-dichlorodiethylamine hydrochloride was 0.45 in the same condition of developing. The spot at Rf 0.12 was assumed to be that of the dimerization product of the substance of Rf 0.45.

Determination of Cl^- Liberation and Thiosulfate Consumption in Sodium Hydrogencarbonate-buffered Solution——Titration was carried out by the procedure completely analogous to those described in a previous report.[5]

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4) H. Satoh : Yakugaku Zasshi, **72**, 74 (1952).

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

The authors wish to express their gratitude to Prof. T. Yoshida, Dr. H. Satoh, and Dr. H. Imamura for their advices and collaboration especially in the biological screening experiments. A part of this work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education and from the Ministry of Health and Welfare, to which the authors' thanks are due.

Summary

Several derivatives of N-(2-substituted ethoxy)-2,2′-dichlorodiethylamine were prepared and tested for their latent antitumor activity against Yoshida sarcoma. Mode of activation of these compounds was also discussed.

(Received December 10, 1960)

UDC 615.771.7:547.233'222

109. Morizo Ishidate,[*2] Yoshio Sakurai,[*2] and Ken-ichi Sawatari[*3] : Studies on Carcinostatic Substances. XXXVII.[*1] Preparation of Derivatives of Nitrogen Mustard having Metal Chelate-forming Activity.

(*Iatrochemical Institute of Pharmacological Research Foundation*[*2]
and Yoshitomi Pharmaceutical Co., Ltd.[*3])

The most important problem is to improve specificity of action of derivatives of simple nitrogen mustard on tumor by modification of the molecular structure. One probable method for this purpose may be the control or restriction of reactivity of the two functional groups of this compound by some means and, from this point of view, copper chelate formation of amino acid derivatives having two 2-chloroethyl groups was investigated.

Needless to say, the reactivity of this functional group depends on the property of lone-pair electrons of nitrogen, which should change when the electrons participate in formation of a chelate. Therefore, it was expected that such a compound in a form of chelate might behave as an antitumor agent having an improved chemotherapeutic index or a long-lasting effect.

The compounds prepared and discussed in this work are shown *en bloc* in Table I and their synthetic procedures are shown in Chart 1.

The chelate-forming activity of 2,2′-dichlorodiethylamine derivative (No. 602) was found to be nearly of the same order as that of the derivative of 2-(2-chloroethylamino)-acetic acid (No. 652) by determination of stability constants of their copper chelates, data of which are listed in Table II.

As will be mentioned below, the derivatives of ethylenediamine have greater tendency to form a chelate with copper ion than those of propylenediamine. The reaction of No. 601 in an aqueous solution is supposed to proceed as shown in Chart 2.

Alkylating velocity depends on the concentration of the aziridinium intermediate (III), which is necessarily dependent on the stability of the chelate in the reaction solution and,

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[*1] Part XXXVI : This Bulletin, **9**, 676 (1961).

[*2] Designation now changed to Cancer Chemotherapy Section, Sasaki Institute. 26 Nishigahara 1-Chome, Kita-ku, Tokyo (石舘守三, 桜井欽夫).

[*3] Yoshitomi-machi, Chikujo-gun, Fukuoka-ken (猿渡健市).