CHEMICAL
&
PHARMACEUTICAL BULLETIN

Vol. 9 No. 5 May 1961

UDC 615.771.7 : 547.233'222

62. Isao Aiko : Studies on Carcinostatic Substances. XXXIII.*2 Preparation of Derivatives of Nitrogen Mustard of Strongly Basic Character.

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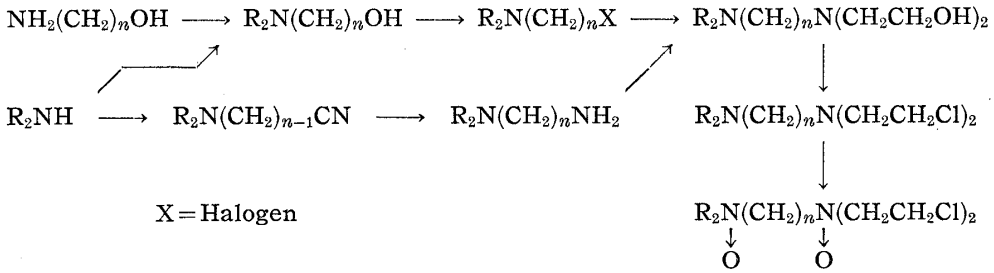
In a previous communication of this series,[1] it was demonstrated that introduction of carboxyl group into the molecule of nitrogen mustard enhanced in some cases a margin of safety, which is usually not large enough among the original compounds. In cases of these derivatives having acid group, especially derivatives of α-amino acid, the free bases are likely to be soluble in physiological liquids due to their amphoteric character. On the contrary, most of hydrochlorides of derivatives of nitrogen mustard liberate the insoluble free bases in a nearly neutral aqueous medium.

For this reason, it has been anticipated that the derivatives which have strongly basic cation group on one side of a molecule might also have a margin of safe dose as large as that of amino acid derivatives, because the former should also remain as soluble cations in physiological liquids like blood and lymph.

From this aspect, preparation of a series of derivatives of N,N-dialkyl-N',N'-bis(2-chloroethyl)polymethylenediamine was undertaken and a discussion on their chemical and biological properties is presented in this paper.

A few compounds belonging to this series, viz. N,N-dimethyl-N',N'-bis(2-chloroethyl)-ethylenediamine N-methochloride and N,N-diethyl-N',N'-bis(2-chloroethyl)ethylenediamine N,N'-dioxide, were already prepared in 1953[2] and proved to have a prominent effect on Yoshida sarcoma.

The processes of synthesis of a series of compounds presented in this paper are as follows :



Most of these dihydroxyl intermediates are colorless or faintly yellow liquid of high viscosity and decomposed more or less even in a highly reduced atmosphere. By chlo-

*1 Yoshitomi-machi, Chikujo-gun, Fukuoka-ken (愛甲軍雄).

*2 This paper constitutes a part of a series entitled "Studies on Carcinostatic Substances" by M. Ishidate and Y. Sakurai. Part XXXII : This Bulletin, 8, 1052 (1960).

1) M. Izumi : This Bulletin, **3**, 275 (1954).

2) M. Ishidate, Y. Sakurai, M. Izumi : *Ibid.*, **1**, 297 (1953).

rination of the intermediates, the products were usually accompanied with some amount of methanol-insoluble by-product of unknown property. The free bases of the final products were highly reactive and purification by distillation was not successful because of unavoidable accompaniment of polymerization.

Oxidation of the chlorinated tertiary diamines to the N,N′-dioxides was carried out according to the procedure already reported.[2)]

The formula of compounds, their melting points, and their anti-tumor effect against Yoshida sarcoma are summarized in Table I.

Table I.

Compd. No.	Compound	Dihydrochloride m.p. (°C)	Picrate m.p. (°C)	(mg./kg.) LD_{50}	MTD[a)]	MED[b)]
	R- : $-N(CH_2CH_2Cl)_2$.					
541	$CH_2=CHCH_2\rangle^{CH_3}N-CH_2CH_2-R$		124～125°	37.5	25	1
530	$CH_2\langle^{CH_2-CH_2}_{CH_2-CH_2}\rangle CH\rangle^{CH_3}N-CH_2CH_2-R$	88～89°	127°	30	10	0.5
506	$\left(CH_2\langle^{CH_2-CH_2}_{CH_2-CH_2}\rangle CH\right)_2 NCH_2CH_2-R$	162～163°	173～174°	30	10	0.1
517	$C_6H_5CH_2\rangle^{CH_3}NCH_2CH_2-R$	126～127°		30	10	0.5
509	$(C_6H_5-CH_2)_2NCH_2CH_2-R$	187～188°		75	50	5
478	$O\langle^{CH_2-CH_2}_{CH_2-CH_2}\rangle NCH_2CH_2-R$	176～179°		30	10	5
515	$C_6H_5-CH_2-N\langle^{CH_2-CH_2}_{CH_2-CH_2}\rangle NCH_2CH_2-R$	263～265°[c)]		75	50	1
516	$C_6H_5\rangle^{C_2H_5}NCH_2CH_2-R$		167～168°	30	10	0.5
519	$(CH_3)_2NCH_2CH_2CH_2-R$	169～170°	141～142°	0.75	0.5	0.01
483	$(C_2H_5)_2NCH_2CH_2CH_2-R$	179～180°		3.75	0.1	0.05
484	N,N′-dioxide of No. 483	176～177°[d)]		375	100	10
492	$(C_6H_5-CH_2)_2NCH_2CH_2CH_2-R$		141～142°	3.0	1.0	0.5
487	N,N′-dioxide of No. 492		127～128°	375	250	5
477	$(C_2H_5)_2NCH_2CH_2CH_2CH_2-R$	174～175°	122～123°	3.0	1.0	0.05
543	N,N′-dioxide of No. 477		137～138°	75	50	0.5
486	$(C_2H_5)_2NCH_2CH_2CH_2CH_2CH_2-R$		149～150°	3.0	1.0	0.05
542	N,N′-dioxide of No. 486		116～117°[e)]	175	100	5

a) Maximum tolerance dose (rat, intraperitoneal).
b) Minimum effective dose (Yoshida sarcoma rat, intraperitoneal) (cf. Gann, **44**, 342 (1953); **45**, 484 (1954)).
c) Trihydrochloride, decompn. *d*) Decompn. *e*) Monohydrate.

To sum up these data, N,N-dialkyl-N′,N′-bis(2-chloroethyl)polymethylenediamines showed in general a wider margin of safety than N-methyl-bis(2-chloroethyl)amine (HN_2) and, especially, the compounds of ethylenediamine series appeared to have a very large chemotherapeutic index (LD_{50}/MED), as seen in Table I. Out of them, No. 506 is the best in aspect of the index and Nos. 515, 516, 517, and 530 exhibit higher or at least the same values of index than that of N-methyl-bis(2-chloroethyl)amine N-oxide (HN_2-O).

Analytical investigation was also undertaken with these compounds in order to find a relationship, if any, between the biological activity, and chemical and physical properties. For instance, rates of Cl^- and H^+ liberation in an aqueous solution, thiosulfate uptake, surface tension of solution, partition coefficient between benzene and water, and apparent dissociation indices of bases were determined, the data of which are listed in Tables II and III.

Of the dissociation indices of a series of derivatives of N,N-diethyl-N′,N′-bis(2-chloroethyl)polymethylenediamine, $pK'b_2$ of the derivative of ethylenediamine, No. 220, is the

Table II. $(C_2H_5)_2N'-(CH_2)_n-N^2(CH_2CH_2Cl)_2$

No.	n	MEC[a] mM	MED mg./kg.	LD_{50} mg./kg.	$pK'b_1$[b]	$pK'b_2$[b]	Cl^- liberation[c] (%)		H^+ liberation[d] (%)		S_2O_3 uptake[e] (%)		Partition coefficient[f]	Surface tension[g] (dyne/cm.)
							10 min.	60 min.	10 min.	60 min.	10 min.	60 min.		
486	5	2.5×10^{-4}	0.05	3.0	4.3	8.4	49	81	5.3	52	80.5	53	0.52	70.7
483	3	5×10^{-4}	0.05	3.75	4.3	9.5	52	94	6.4	78	49.7	16	0.65	71.6
477	4	2.5×10^{-3}	0.05	3.0	4.3	8.6	63	80	15	59	54	21	0.47	70.4
220	2	2.5×10^{-2}	1.0	8.0	4.5	11.7	41	71	47	64	13	7	1.68	66.3
24	(HN_2)[h]	2.5×10^{-4}	0.05	1.6	—	6.2	52	83	4	25	61	52	0.54	69.3

a) Minimum effective concentration on *in vitro*-cultured Yoshida sarcoma cells (cf. This Bulletin, **7**, 873 (1959)).
b) Apparent dissociation index of amine at 29°.
c) Sample, 0.02 mmole/cc. $NaHCO_3$, 0.8 mmole/cc. at 37°.
d) Sample, 0.1 mmole/cc. at 37°. The solution was continuously titrated with 0.1N NaOH to keep its reaction at pH 8.3.
e) Sample, 0.02 mmole/cc. $Na_2S_2O_3$, 0.08 mmole/cc. $NaHCO_3$, 0.16 mmole/cc. The mixture of the sample and $NaHCO_3$ was kept at 37° for 10 or 60 min. and then $Na_2S_2O_3$ was added. The whole mixture was titrated with 0.1N I_2 after 120 min. incubation at 25°.
f) Between benzene and H_2O (pH 7.3) at 29°.
g) 0.2M solution at 20°.
h) N-Methyl-bis(2-chloroethyl)amine.

Table III. $(R)_2{}^1N-CH_2CH_2-{}^2N(CH_2CH_2Cl)_2\cdot 2HCl$

No.	Formula; $(R)_2N-$	MEC mM	MED mg./kg.	LD_{50} mg./kg.	$pK'b_1$	$pK'b_2$	Cl^- liberation (%)		H^+ liberation (%)		S_2O_3 uptake (%)		Partition coefficient	Surface tension (dyne/cm.)
							10 min.	60 min.	10 min.	60 min.	10 min.	60 min.		
506	$\left(CH_2(CH_2-CH_2)_2CH-\right)_2N-$	2.5×10^{-3}	0.1	30	6.1	11.8	29[c]	76[c]	9[c]	55[c]	23[c]	7[c]	22.5	55.7
220	$(C_2H_5)_2N-$	2.5×10^{-2}	1.0	8	4.5	11.7	44	73	47	55	13	7	1.68	66.3
509	$(C_6H_5CH_2)_2N-$	1×10^{-2}	5.0	75	8.7	11.9	49[c]	73[c]	2[c]	26[c]	33[c]	2[c]	21.4	52.2
478	$O(CH_2-CH_2)_2N-$	2.5×10^{-1}	5.0	30	7.3	11.9	55	67	32	41	6	1	0.88	71.3
515	$C_6H_5-CH_2-N(CH_2-CH_2)_2N-$[a]	1×10^{-1}	1.0	75										
24	HN_2[b]	2.5×10^{-4}	0.05	1.6		6.2	52	83	4	25	61	52	0.54	69.3

a) Trihydrochloride.
b) Reference compound, N-methyl-bis(2-chloroethyl)amine hydrochloride.
c) 75% MeOH solution.
Note : The same abbreviations are used as those in Table II.

largest, but these values decrease as the central carbon chain becomes longer. Although basicity of bis(2-chloroethyl)amino group should be under the influence of -I effect of diethylamino group on the other side of the molecule in all cases, the effect seems to be strong enough only in the case of ethylenediamine derivatives. In fact, the slight effect is still observed among the higher homologs as seen in Table II and even $pK'b_2$ of No. 486 was found to be somewhat larger than that of nitrogen mustard.

According to Cohen,[3] there is a rule among reactions of derivatives of nitrogen mustard that the smaller the value of pK'b is, the larger will be the velocity of ethyleneimmonium-intermediate formation in a dilute aqueous solution. Two possible reactions, viz. alkylation and hydrolysis, of this three-membered intermediate are believed to be competitive with each other and, so far as known from thiosulfate uptake of the aged solution of these compounds demonstrated in Figs. 1, 2, and 3, a comparative proportion of alkylation to hydrolysis increases gradually as the length between two nitrogen atoms becomes longer.

The result obtained above seems to suggest a possibility of finding the most favorable anti-tumor derivative of nitrogen mustard by controlling the alkylating reactivity with introduction of a strong basic amino group on one side of the molecule at a certain distance from (2-chloroethyl)amino group.

On the other hand, there was found no decisive correlation between anti-tumor activity and surface tension or partition coefficient. It must also be noted here that the partition coefficient in the table means only an approximate value, because prompt transformation of the compounds into ethyleneimmonium forms in a neutral aqueous solution cannot be avoided even in the course of measurement.

The similar data of experiment with a series of derivatives of N,N-dialkyl-N',N'-bis(2-chloroethyl)ethylenediamine are presented in Table III.

The values of $pK'b_2$ of these compounds appeared to be nearly the same, despite the fact that $pK'b_1$ values varied as the substituents at N^1 differed. It is worth noting, however, that the values of thiosulfate uptake of the series of compounds are quite different from each other and, therefore, it is supposed that $pK'b_2$ value is not an exclusive factor which influences alkylating activity of a compound.

A competitive ratio of alkylation to hydrolysis of the ethyleneimmonium intermediate may perhaps be another factor of importance and this ratio of competition may be approximately assumed from the values of thiosulfate uptake of the aged solution of each compound, values of which are shown in Figs. 1, 2, 3, and 4.

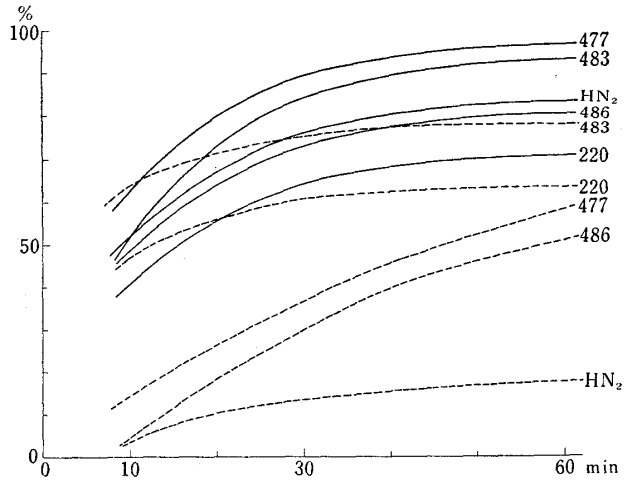


Fig. 1. Rate of Cl^- and H^+ Liberations of a Series of Compounds (37°C, Hydrogencarbonate Buffer)

—— Cl^- liberation
------ H^+ liberation

3) B. Cohen, E. R. Van Artsdalen, J. Harris : J. Am. Chem. Soc., **70**, 281 (1948).

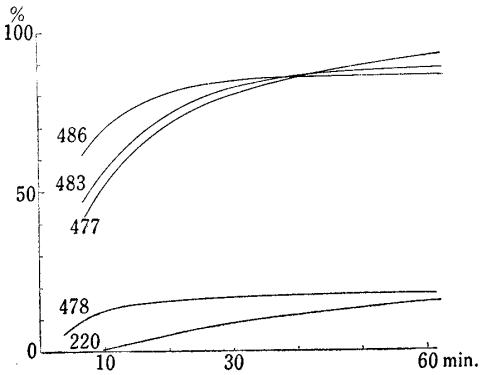


Fig. 2. Rate of Thiosulfate Uptake of a Series of Compounds
(25°, hydrogencarbonate buffer)

$(C_2H_5)_2N(CH_2)_nN(CH_2CH_2Cl)_2$
220 : $n=2$
483 : $n=3$
477 : $n=4$
486 : $n=5$
478 : 

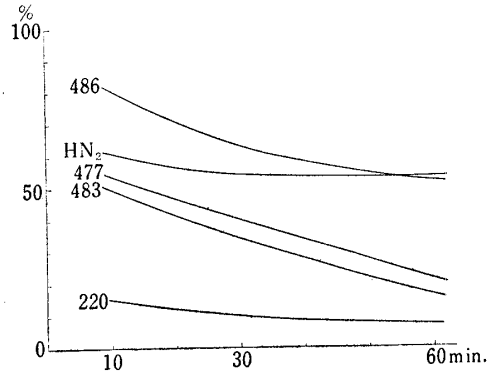


Fig. 3. Rate of Thiosulfate Uptake of Aged Solution of a Series of Compounds
(37°, hydrogencarbonate buffer)

$(C_2H_5)_2N\text{-}(CH_2)_nN(CH_2CH_2Cl)_2$
220 : $n=2$
483 : $n=3$
477 : $n=4$
486 : $n=5$

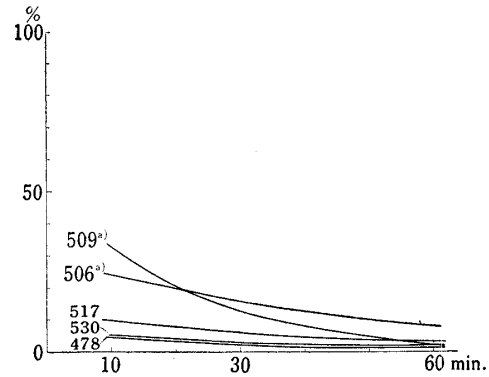
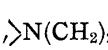
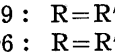
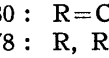


Fig. 4. Rate of Thiosulfate Uptake of Aged Solution of a Series of Compounds
(37°, hydrogencarbonate buffer)


509 : $R=R'=C_6H_5CH_2\text{-}$
506 : 
517 : $R=CH_3\text{-}$, $R'=C_6H_5\text{-}CH_2\text{-}$
530 : $R=CH_3\text{-}$, 
478 : R, R′=morpholinyl

a) Methanol (75%) used as solvent.

The stronger anti-tumor activity of No. 477 than No. 220 is due to the higher stability of the ethyleneimmonium intermediate of the former in a neutral solution which does not contain thiosulfate, as demonstrated in Table II and Fig. 3.

The compounds Nos. 506 and 509 in Table III were found to have a very large partition coefficient. As it is supposed that solvation of a molecule with polar solvent accelerates the velocity of carbonium ion formation, it is easy to think that the velocity of Cl^- liberation of the highly lipophilic compounds such as Nos. 506 or 509 is comparatively small. Notwithstanding the fact, No. 506 was proved to be very active anti-tumor agent on account of its high stability of its intermediate in a solution as seen in Fig. 4.

In conclusion, anti-tumor activity of these compounds seems to depend chiefly on concentration of ethyleneimmonium intermediate in the reacting milieu and partly on competitive ratio of alkylation to hydrolysis. A concentration of the intermediate is also controlled by its yielding velocity and stability against hydrolysis, and, as discussed above, the yielding velocity of the intermediate is also influenced by value of $pK'b_2$.

When these tertiary diamines were oxidized to the N,N′-dioxides, anti-tumor effect

decreased markedly, although toxicity was diminished to some extent as anticipated. Several N,N-dioxides are shown in Tables I and IV.

Table IV.

No.	Compound (dihydrochloride)	MEC m*M*	MED mg./kg.	LD_{50} mg./kg.	$pK'b_1$	$pK'b_2$	Liberation Cl^- : 60 min.	Liberation H^+ : 60 min.	S_2O_3 uptake : 60 min.	Partition coefficient	$\pi^{a)}$
221	$(C_2H_5)_2NCH_2CH_2N(CH_2CH_2Cl)_2$ (↓O, ↓O)	2.5×10^{-2}	4	150	7.9	12.1	61	52	6	0	−0.539
484	$(C_2H_5)_2NCH_2CH_2CH_2N(CH_2CH_2Cl)_2$ (↓O, ↓O)	2.5×10^{-2}	10	375	8.9-	11.1	48	27	27	0	−0.460

a) Half-wave potential at 25°, pH 3.5, $10^{-4}M$.
Note : The same abbreviations are used as those in Table II.

For example, Nos. 221 and 484 seemed to have a very low toxicity but little effectiveness, inspite of the fact that the values of $pK'b_2$ of these compounds are nearly the same as those of the original tertiary diamines.

Contrary to anticipation, they exhibited anti-tumor activity against the *in vitro*-cultured Yoshida sarcoma cells even at a small population as 10^5 cells/cc. although the activity was very poor and minimum effective concentration (MEC) of these compounds on the tumor cells *in vitro* did not vary with increase of cell population. That is not the case with N-methyl-bis(2-chloroethyl)amine N-oxide (HN_2–O) as described in a previous report.[4] The fact might sugggest that the N-oxide-masking of these compounds is far more easily removed by the cells than in the case of N-methyl-bis(2-chloroethyl)amine N-oxide, because half-wave potentials of Nos. 221 and 484 by polarography were determined respectively as −0.539 V and −0.460 V (vs. S.C.E., pH 3.5, 25°), which are more positive than that of N-methyl-bis(2-chloroethyl)amine N-oxide (−0.78 V, vs. N.C.E., pH 3.5, 25°).[5]

Experimental

A) Preparation of N,N-Dialkyl-N′,N′-bis(2-chloroethyl)ethylenediamine

2-(N-Methylallylamino)ethanol——Allyl bromide (60.5 g.) was added in drops into a mixture of N-2-(methylamino)ethanol[6] (41 g.) and benzene (300 cc.) with stirring at 40° and the mixture was kept at the same temperature for 1.5 hr. The reaction mixture was shaken with dil. NaOH and the benzene layer was dried and evaporated to dryness. The residue was distilled *in vacuo* and a colorless liquid of high viscosity, $b.p_{50}$ 88~92°, was obtained. Yield, 32 g.

N-(2-Chloroethyl)-N-methylallylamine——Into a mixture of 2-(N-methylallylamino)ethanol (55 g.) and benzene (50 cc.), a mixture of $SOCl_2$ (68 g.) and benzene (100 cc.) was added in drops with stirring at 30~40° and the whole mixture was refluxed for 2 hr. After cool, the separated crystals were collected and added to dil. NaOH. An separated oily layer was extracted with benzene, dried, and distilled *in vacuo*. Colorless liquid, $b.p_{35}$ 63~66°. Yield, 30 g.

N-Allyl-N-methyl-N′,N′-bis(2-hydroxyethyl)ethylenediamine——A mixture of N-(2-chloroethyl)-N-methylallylamine (30 g.), diethanolamine (22 g.), K_2CO_3 (22 g.), and EtOH (100 cc.) was refluxed with stirring for 20 hr. The insoluble inorganic substances were removed by filtration and the filtrate was distilled *in vacuo* after the solvent was evaporated. Colorless and viscous liquid, $b.p_6$ 135~140°. Yield, 22 g.

N-Allyl-N-methyl-N′,N′-bis(2-chloroethyl)ethylenediamine (No. 541)——A mixture of N-allyl-N-methyl-N′,N′-bis(2-hydroxyethyl)ethylenediamine (22 g.) and benzene (30 cc.) was added in drops into a mixture of $SOCl_2$ (33 g.) and benzene (40 cc.) with stirring at 30~40°, the whole mixture was warmed gently, and refluxed for 1.5 hr. After cool. the benzene layer was evaporated. The dark residue was dissolved in a small quantity of H_2O, shaken with activated charcoal, and added with picric acid solution.

4) M. Ishidate, Y. Sakurai, H. Imamura : This Bulletin, **8**, 449 (1960).
5) I. Aiko : *Ibid.*, **1**, 335 (1953).
6) L. Knorr, H. Mathes : Ber., **31**, 1069 (1898).

Dipicrate : m.p. 124~125° (Me_2CO). *Anal.* Calcd. for $C_{22}H_{26}O_{14}N_8Cl_2$: C, 37.89; H, 3.76; N, 16.06. Found : C, 38.07; H, 3.86; N, 15.77.

The dihydrochloride was not obtained as crystals.

N-Cyclohexyl-N-methyl-N′,N′-bis(2-hydroxyethyl)ethylenediamine——Viscous liquid, b.p_3 180~185°.

N-Cyclohexyl-N-methyl-N′,N′-bis(2-chloroethyl)ethylenediamine (No. 530)——Dihydrochloride: m.p. 88~89° (EtOH-AcOEt=1:2). *Anal.* Calcd. for $C_{13}H_{28}N_2Cl_4$: C, 44.08; H, 7.97; N, 7.91. Found: C, 44.20; H, 7.90; N, 7.90.

N,N-Dicyclohexyl-N′,N′-bis(2-hydroxyethyl)ethylenediamine——Viscous liquid, b.p. 200~203°.

N,N-Dicyclohexyl-N′,N′-bis(2-chloroethyl)ethylenediamine (No. 506)——Dihydrochloride : m.p. 162~163° (EtOH). *Anal.* Calcd. for $C_{18}H_{36}N_2Cl_4$: C, 51.19; H, 8.59; N, 6.63. Found : C, 51.36; H, 8.85; N, 6.60. Dipicrate : m.p. 173~174° (Me_2CO).

N-Benzyl-N-methyl-N′,N′-bis(2-hydroxyethyl)ethylenediamine——Viscous liquid, b.p_5 155~156.5°.

N-Benzyl-N-methyl-N′,N′-bis(2-chloroethyl)ethylenediamine (No. 517)——Dihydrochloride, m.p. 126~127° (EtOH-AcOEt=1:2). *Anal.* Calcd. for $C_{14}H_{24}N_2Cl_4$: C, 46.42; H, 6.68; N, 7.74. Found : C, 46.52; H, 6.72; N, 7.42.

N,N-Dibenzyl-N′,N′-bis(2-hydroxyethyl)ethylenediamine——Viscous liquid, b.$p_{0.4}$ 215~222°.

N,N-Dibenzyl-N′,N′-bis(2-chloroethyl)ethylenediamine (No. 509)——Dihydrochloride : m.p. 187~188° (EtOH-AcOEt). *Anal.* Calcd. for $C_{20}H_{28}N_2Cl_4$: C, 54.81; H, 6.44; N, 6.39. Found : C, 54.71; H, 6.17; N, 6.66.

4-[2-Bis(2-hydroxyethyl)aminoethyl]morpholine——Viscous liquid, b.p_9 195~200°.

4-[2-Bis(2-chloroethyl)aminoethyl]morpholine (No. 478)——Dihydrochloride : m.p. 176~179°. *Anal.* Calcd. for $C_{10}H_{22}ON_2Cl_4$: C, 36.60; H, 6.76; N, 8.54. Found : C, 36.89; H, 6.60; N, 8.37.

1-Benzyl-4-[2-bis(2-hydroxyethyl)aminoethyl]piperazine——Viscous liquid, b.p_5 220~224°. Trihydrochloride, m.p. 220° (decomp.). *Anal.* Calcd. for $C_{17}H_{32}O_2N_3Cl_3$: N, 10.08. Found : N, 9.90.

1-Benzyl-4-[2-bis(2-chloroethyl)aminoethyl]piperazine (No. 515)——Trihydrochloride : m.p. 263~265° (decomp.). *Anal.* Calcd. for $C_{17}H_{30}N_3Cl_5$: C, 45.00; H, 6.67; N, 9.26. Found : C, 45.33; H, 6.96; N, 9.26.

B) Preparation of N,N-Dialkyl-N′,N′-bis(2-chloroethyl)polymethylenediamine

3-(Diethylamino)-1-propanol——Into a mixture of 3-amino-1-propanol (75 g.), NaOH (160 g.), and H_2O (200 cc.), Et_2SO_4 (370 g.) was added dropwise with stirring at 40°. The whole mixture was heated gradually up to 90° during 1 hr. and then cooled. The separated oil was extracted in benzene. The aqueous layer was saturated with K_2CO_3 and extracted with benzene. Two benzene extracts were combined and dried over anhyd. K_2CO_3. The solvent was then evaporated and the residue was distilled *in vacuo*. Colorless liquid, b.p_{20} 82~85°. Yield, 69 g.

N,N-Diethyl-3-chloropropylamine[7]——Colorless liquid, b.p_{750} 160°. Yield, 45 g.

N,N-Diethyl-N′,N′-bis(2-hydroxyethyl)-1,3-propanediamine——i) A mixture of N,N-diethyl-3-chloropropylamine (45 g.), diethanolamine (29 g.), 1-propanol (100 cc.), and K_2CO_3 (25 g.) was refluxed with stirring for 20 hr. After filtration the solvent was evaporated from the filtrate and the residue was distilled *in vacuo*. Faint yellow viscous liquid, b.p_8 185~198°. Yield, 47 g. A Schimon test for secondary amine was negative with this substance.

ii) A solution of ethylene oxide (66 g.) dissolved in H_2O (100 cc.) was added dropwise into a mixture of N,N-diethyl-1,3-propanediamine[8] (b.p. 165~170°) (75 g.) and H_2O (100 cc.). The whole mixture was left standing in a sealed bottle overnight at room temperature. Excess of the reagent and solvent were distilled off and the residue was treated as in case of (i). The crude product was used as such for the next process without purification.

N,N-Diethyl-N′,N′-bis(2-chloroethyl)-1,3-propanediamine (No. 483)——Into a mixture of N,N-diethyl-N′,N′-bis(2-hydroxyethyl)-1,3-propanediamine (75 g.) and $CHCl_3$ (150 cc.), $SOCl_2$ (32 g.) was added dropwise with stirring at 30~40°. The mixture was kept at 60° for 2 hr. with continuous stirring. After distillation of solvent, the residue crystallized on addition of EtOH, m.p. 179~180° (EtOH). *Anal.* Calcd. for $C_{11}H_{26}N_2Cl_4$: C, 40.26; H, 7.99; N, 8.54. Found : C, 40.25; H, 8.19; N, 8.71.

N,N-Dimethyl-N′,N′-bis(2-hydroxyethyl)-1,3-propanediamine——Colorless and viscous liquid, b.p_7 164~175°.

N,N-Dimethyl-N′,N′-bis(2-chloroethyl)-1,3-propanediamine (No. 519)——Dihydrochloride : Very hygroscopic, m.p. 169~170° (EtOH-Me_2CO). *Anal.* Calcd. for $C_9H_{22}N_2Cl_4$: C, 36.01; H, 7.39. Found: C, 36.19; H, 7.17.

N,N-Dibenzyl-N′,N′-bis(2-hydroxyethyl)-1,3-propanediamine——Colorless and viscous liquid, b.$p_{0.1}$ 210~220.

N,N-Dibenzyl-N′,N′-bis(2-chloroethyl)-1,3-propanediamine——Dipicrate : Yellow crystals, m.p.

7) R. R. Adams, F. C. Whitmore : J. Am. Chem. Soc., **67**, 735 (1945).
8) F. C. Whitmore, *et al.* : *Ibid.*, **66**, 725 (1944).

141～142°. *Anal.* Calcd. for $C_{33}H_{34}O_{14}N_8Cl_2$: C, 47.32; H, 4.09; N, 13.35. Found : C, 47.61; H, 4.09; N, 13.03.

4-Bromobutyl Phenyl Ether——Prepared according to the process for 3-bromopropyl phenyl ether.[9] Colorless liquid, b.p_{18} 150～158°. Yield, 56%.

N,N-Diethyl-4-phenoxybutylamine——Prepared according to the process of N,N-diethyl-3-phenoxypropylamine.[10] Colorless liquid, b.p_{21} 150～159°. Yield, 82%.

N,N-Diethyl-4-bromobutylamine——A mixture of N,N-diethyl-4-phenoxybutylamine (89 g.) and 48% HBr (100 cc.) was heated at 180～220° for 5 hr. The reaction mixture was distilled, the residue was added with 48% HBr (100 cc.), and heated. The same procedure was repeated at least 3 times in total. The final residue was dissolved in H_2O (100 cc.) and shaken with activated charcoal. The mixture was evaporated *in vacuo* the residue was made alkaline with 50% NaOH and extracted twice with Et_2O (50 cc.). The solvent was distilled off and the residue was fractionated by distillation *in vacuo*. Colorless liquid, b.p_6 69～75°. Yield, 46 g. It was used immediately in the next reaction. For preservation, it is necessary to convert it to hydrochloride because of instability of the free amine.

4-Diethylaminobutyronitrile[8]——Colorless liquid, b.p_{21} 101～105°. Yield, 76.5%.

N,N-Diethyl-1,4-butanediamine[8]——Colorless liquid, b.p_{30} 95～100°. Yield, 76.5%.

N,N-Diethyl-N′,N′-bis(2-hydroxyethyl)-1,4-butanediamine——Faint yellow, viscous liquid, b.p_4 160～170°. Yield, 62%.

N,N-Diethyl-N′,N′-bis(2-chloroethyl)-1,4-butanediamine (No. 477)——Dihydrochloride : m.p. 174～175° (decomp.). *Anal.* Calcd. for $C_{12}H_{28}N_2Cl_4$: C, 42.11; H, 8.25; N, 8.19. Found : C, 42.30; H, 8.14; N, 8.44.

N,N-Diethyl-N′,N′-bis(2-hydroxyethyl)-1,5-pentanediamine——Faint yellow, viscous liquid, b.p_4 181～190°.

N,N-Diethyl-N′,N′-bis(2-chloroethyl)-1,5-pentanediamine (No. 486)——Dipicrate : m.p. 149～150° (MeOH). *Anal.* Calcd. for $C_{25}H_{34}O_{14}N_8Cl_2$: C, 40.49; H, 4.62; N, 15.11. Found : C, 40.62; H, 4.35; N, 14.90.

C) Preparation of N,N-Dialkyl-N′,N′-bis(2-chloroethyl)polymethylenediamine N,N′-Dioxide

N,N-Diethyl-N′,N′-bis(2-chloroethyl)-1,3-propanediamioe N,N′-Dioxide (No. 484)——N,N-Diethyl-N′,N′-bis(2-chloroethyl)-1,3-propanediamine hydrochloride (32 g.) was dissolved in H_2O, made alkaline with dil. NaOH, and extracted immediately with benzene (100 cc.). The extract was added in drops into a solution of AcOOH, prepared from 30% H_2O_2 (65 g.) and Ac_2O (65 g.), with stirring at about 10°. The reaction mixture was kept for 3 hr. with frequent shaking at the same temperature. After cool, conc. HCl was added with vigorous stirring and the benzene layer was removed. The H_2O layer was evaporated to dryness in a reduced atmosphere below 30° and the residue was chilled in ice until it became crystalline. White crystals, m.p. 176～177° (decomp.). *Anal.* Calcd. for $C_{11}H_{26}O_2N_2Cl_4$: C, 36.68; H, 7.28; N, 7.78. Found : C, 36.51; H, 7.20; N, 7.62.

N,N-Dibenzyl-N′,N′-bis(2-chloroethyl)-1,3-propanediamine N,N′-Dioxide (No. 487)——Dipicrate: m.p. 127～128°. *Anal.* Calcd. for $C_{33}H_{34}O_{16}N_8Cl_2$: C, 45.58; H, 3.94. Found : C, 45.87; H, 3.92.

N,N-Diethyl-N′,N′-bis(2-chloroethyl)-1,4-butanediamine N,N′-Dioxide (No. 543)——Dipicrate, m.p. 137～138°. *Anal.* Calcd. for $C_{24}H_{32}O_{16}N_8Cl_2$: C, 38.00; H, 4.25; N, 14.77. Found : C, 37.90; H, 4.17; N, 14.49.

N,N-Diethyl-N′,N′-bis(2-chloroethyl)-1,5-pentanediamine N,N′-Dioxide (No. 542)——Dipicrate monohydrate : m.p. 116～117°. *Anal.* Calcd. for $C_{25}H_{36}O_{17}N_8Cl_2$: C, 37.93; H, 4.33; N, 14.16. Found: C, 37.89; H, 4.29; N, 14.04.

D) Determination of Chemical and Physical Properties

Measurement of Liberated Cl^-——A sample to be tested (0.1 mmole) and $NaHCO_3$ (0.6 mmole) were dissolved in H_2O (5 cc.) and incubated at 37° for the necessary period. The liberated Cl^- was titrated potentiometrically with 0.01*N* $AgNO_3$ using a glass and silver electrodes, according to the method of Golumbic, *et al.*[11]

Measurement of Liberated H^+——A sample to be tested (0.1 mmole) was dissolved in H_2O (5 cc.) and continuously titrated with 0.01*N* NaOH at 37°, using a glass-electrode pH-meter so as to keep the solution constantly at pH 8.3. The volume of 0.01*N* NaOH consumed during titration was regarded as equivalent to the amount of liberated H^+.

Measurement of Thiosulfate Uptake——A sample to be tested (0.5 mmole) and $NaHCO_3$ (2.5 mmole) were dissolved in H_2O (30 cc.) and immediately added with 0.1*N* $Na_2S_2O_3$ (10 cc.). The whole mixture was made up exactly to 50 cc. and incubated at 25°. After the necessary period of incubation, the solution was back-titrated with 0.02*N* I_2.

9) Org. Syntheses, Coll. Vol. I, 425.
10) C. S. Marvel, W. H. Zartman, O. D. Bluthardt : J. Am. Chem. Soc., **49**, 2300 (1927).
11) C. Golumbic, J. S. Fruton, M. Bergmann : J. Org. Chem., **11**, 518 (1946).

Measurement of Thiosulfate Uptake of Aged Solution——A sample to be tested (0.1 mmole) and $NaHCO_3$ (0.8 mmole) were dissolved in H_2O (5 cc.) and incubated at 37° for the necessary length of time. After incubation, it was added with 0.01*N* $Na_2S_2O_3$ (20 cc.) and again incubated at 37° for 10 min. The solution was then back-titrated with 0.01*N* I_2 potentiometrically or with starch indicator. Phosphate buffer should be avoided in this experiment, because these 2-chloroethylamines seemed to form esters with phosphate.

Measurement of Apparent Dissociation Index——A solution of a test sample (0.01*M*) was titrated with 0.01*N* HCl at 29° according to the method described by Yoshimura and Shoji.[12] In case of a very weak base, the determination was carried out by back-titration with 0.01*N* NaOH after addition of excess of 0.01*N* HCl.[13]

Measurement of Partision Coefficient——A sample to be tested (0.01 mole) was dissolved in a buffer at pH 7.3 (30 cc.), over which benzene (30 cc.) was added. The mixture was vigorouly shaken at 30° for 5 min. and the two layers were separated. Nitrogen content of each layer was detemined by micro-Kjeldahl method.

Measurement of Surface Tension——Surface tension of a solution of sample to be tested (0.2*M*) was measured by the drop method[14] at 20°. The du Noüy ring-method[15] gave a too large value in these experiments as Harkins pointed out.[15]

The author is deeply grateful to Prof. M. Ishidate and Dr. Y. Sakurai for their kind guidance throughout the course of this investigation. Thanks are also due to Prof. T. Yoshida and Dr. H. Satoh for their profitable advices. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, which is acknowledged with gratitude.

Summary

Derivatives of N,N-dialkyl-N′,N′-bis(2-chloroethyl)polymethylenediamine and some of their N,N′-dioxides were synthesized. These compounds are supposed to remain as cations even in physiological liquids contrary to N-methyl-bis(2-chloroethyl)amine, because the dialkylamino group in these molecules is far more strongly basic than bis(2-chloroethyl)-amino group. Relationship between anti-tumor activity and chemical and physical characteristics of these compounds are also discussed.

(Received July 14, 1960)

12) T. Yoshimura, R. Shoji : "pH no Riron to Sokuteihō," 62 (1941). Maruzen Co., Tokyo.
13) K. Nakanishi : "Yukikagaku no Shimpo," Vol. 12, 22 (1954). Kyōritsu Shuppan Co., Tokyo.
14) T. Ono, T. Sasaki : "Jikken Kagaku Kōza," **7**, 11 (1956). Maruzen Co., Tokyo.
15) W. D. Haakins, H. F. Jordan : J. Am. Chem. Soc., **52**, 1756 (1938).