UDC 615.771.7[547.233'222]-06 : 616-006-085

18. Morizo Ishidate,*2 Yoshio Sakurai,*2 and Isao Aiko*3 : Studies on Carcinostatic Substances. XXVI.*1 Preparation of Bis(2-chloroethyl)amine Derivatives and Related N-Oxides.

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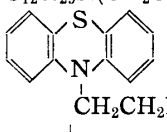
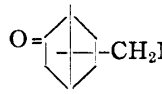
Since a promising activity as an anti-tumor agent was found in N-methyl-bis(2-chloroethyl)amine N-oxide,[1] numerous derivatives of bis(2-chloroethyl)amine have been synthesized and tested[2] as to their anti-tumor activity against Yoshida sarcoma or other experimental tumors.

Out of them, N-isoamyl-, N-benzyl-, and N-(2-ethoxyethyl)-bis(2-chloroethyl)amine N-oxide and N-(2-diethylaminoethyl)-bis(2-chloroethyl)amine were accepted by clinical investigators and proved also effective against human tumor.[3]

Table I.

Compound No.	S	LD_{50} (mg./kg.)	MTD (mg./kg.)	MED (mg./kg.)	MEC (mM)
328	$(CH_3)_3CN(CH_2CH_2Cl)_2$	3	1	0.05	1×10^{-3}
354	Oxide of No. 328	300	200	25	
423	$C_6H_{13}N(CH_2CH_2Cl)_2$	30	10	0.1	5×10^{-3}
—	$C_{12}H_{25}N(CH_2CH_2Cl)_2$[a]				
400		50	10	1	
414		30	10	0.5	6×10^{-3}
430	Oxide of No. 414	30	10	5	
204	$C_2H_5OCH_2CH_2N(CH_2CH_2Cl)_2$[2]	40	20	1	
403	$CH_3OCH_2CH_2N(CH_2CH_2Cl)_2$	0.3	0.1	0.01	5×10^{-3}
404	Oxide of No. 403	7.5	5	1	2.5×10^{-1}
253	$C_4H_9OCH_2CH_2N(CH_2CH_2Cl)_2$	30	10	0.1	1×10^{-2}
255	Oxide of No. 253	80	50	5	1×10^{-1}
471	$C_6H_5OCH_2CH_2N(CH_2CH_2Cl)_2$	7.5	5	0.05	5×10^{-3}
472	Oxide of No. 471	175	100	10	
407	$OHCCH_2N(CH_2CH_2Cl)_2$	3	1	0.01	
406	$(CH_3O)_2CHCH_2N(CH_2CH_2Cl)_2$	200	100	—	
—	Oxide of No. 406[2]				
417	$(C_2H_5O)_2CHCH_2N(CH_2CH_2Cl)_2$	100	50	50	
424	$(C_4H_9O)_2CHCH_2N(CH_2CH_2Cl)_2$	150	100	—	
323	$CH_3COCH_2CH_2N(CH_2CH_2Cl)_2$	3	1	0.1	

a) Data of animal experiment not definite.
LD_{50} Rat (i. p.).
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*.

*1 Part XXV : This Bulletin, 8, 168(1960).
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1) M. Ishidate, T. Yoshida, Y. Sakurai, H. Sato, K. Kobayashi : Proc. Japan Acad., **27**, 493(1951).
2) Y. Sakurai, M. Izumi : This Bulletin, **1**, 297(1953).
3) T. Kurokawa : Gann, **47**, 451(1956).

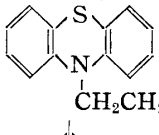
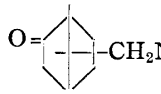
Since then, examinations have been made for new homologs of nitrogen mustard or its N-oxide which might be more effective for practical use. The compounds prepared and tested hitherto are shown in Table I.

Among these compounds, Nos. 328, 423, 403, and 323 have already been reported respectively by Bodenstein, Burchenal, Landing, and Wilson,[4] but the synthetic procedure of each compound has not been described.

Most of the compounds were prepared according to the process which was already stated in the preceding paper of this series,[2] but an attempt for preparing N-benzyloxyethyl-bis(2-chloroethyl)amine happened to be unsuccessful, because its corresponding hydroxyl intermediate afforded nothing but benzyl chloride and tris(2-chloroethyl)amine when chlorinated with thionyl chloride. The preparation of No. 323 was first achieved only by the Mannich reaction as described in the Experimental part and the preparation of No. 407 was also successful only by hydrolysis of No. 406, because the hydroxyl intermediate of the former decomposed at the stage of final chlorination in the aforestated routine synthetic procedure.

The hydroxyl intermediates herein used are listed *en bloc* in Table II.

Table II.

Compound No.		b.p. (°C/mm. Hg)	Synthetic procedure
1	$(CH_3)_3CN(CH_2CH_2OH)_2$	132~135/8	B
2	$C_{12}H_{25}N(CH_2CH_2OH)_2$	200~210/4	A, B
3		250~260/0.02	A
4		(Hydrochloride m.p. 218~220 (d.)[2])	B
5	$CH_3OCH_2CH_2N(CH_2CH_2OH)_2$	133~140/4	A
6	$C_4H_9OCH_2CH_2N(CH_2CH_2OH)_2$	154~160/7	A
7	$C_6H_5OCH_2CH_2N(CH_2CH_2OH)_2$	200~210/4	A
8	$(CH_3O)_2CHCH_2N(CH_2CH_2OH)_2$	127~133/4, 165~169/5	A
9	$(C_2H_5O)_2CHCH_2N(CH_2CH_2OH)_2$	138~142/3, 161~165/4	A
10	$(C_4H_9O)_2CHCH_2N(CH_2CH_2OH)_2$	175~176/3	A

A : $RX + HN(CH_2CH_2OH)_2$ B : $RNH_2 + 2CH_2\text{-}CH_2$ (O)

These compounds were usually very viscous and hygroscopic liquids of a high boiling point, as shown in Table II, and their complete purification for elementary analysis was somewhat difficult. Therefore, they were used as such for chlorination without further repetition of distillation.

Concerning the biological activity and toxicity of some of these compounds, a brief report has been published by the present authors.[5] It was proved that a few of these compounds were not only effective but had a large chemotherapeutic index against Yoshida sarcoma, some of which were comparable to that of N-methyl-bis(2-chloroethyl)amine N-oxide.

Compound No. 423 was found to have an extremely large index (LD_{50}/MED=300), which was regarded as the largest among those of the homologs of N-alkyl-bis(2-chloroethyl)amine. A higher homolog, for instance, N-dodecyl-bis(2-chloroethyl)amine, was not suitable for practical use owing to instant separation of its insoluble free base in a dilute aqueous solution.

4) R. B. Ross, E. W. Foltz, P. E. Schwarzentruber, R. B. Ing, J. Klapp : "Literary Survey of Nitrogen Mustards (1957)."
5) M. Ishidate, T. Yoshida, Y. Sakurai, *et al.* : Gann, **46**, 475(1955); **47**, 375(1956).

Compounds Nos. 400 and 414 exhibited fairly large chemotherapeutic indices and also had a highly lipophilic property, but No. 414 did not give tumor spectrum any different from that of N-methyl-bis(2-chloroethyl)amine N-oxide against a series of rat ascites hepatomas, each of which had a varied susceptibility against the latter agent.[6]

In 1953, N-(2-ethoxyethyl)-bis(2-chloroethyl)amine and its N-oxide (No. 204) were synthesized[2] and the latter was proved to be very effective in prolonging life span of Yoshida sarcoma rats.[7] In the present work, a series of their homologs, Nos. 403, 253, 471, 404, 255, and 472, were added, among which No. 403 showed the strongest effect on the tumor cell, but, at the same time, had the highest toxicity on the host. The largest chemotherapeutic index was observed in No. 253.

The N-oxide derivatives of these homologs had less toxicity than those of the corresponding tertiary amines, but did not surpass No. 204 in respect to chemotherapeutic index or any practical effectiveness. It could be noticed that, while bis(2-chloroethyl)-aminoacetaldehyde (No. 407) exhibited high activity, its acetals, Nos. 406 and 417, showed only a slight or no activity.

Experiments are being carried out to test these compounds on a resistant strain of Yoshida sarcoma, which is 100 times less sensitive against N-methyl-bis(2-chloroethyl)-amine N-oxide. The result will be published in due time.

Experimental

Most of the compounds except Nos. 323 and 407 were prepared according to the process described in the preceding paper.[2]

N-*tert*-Butyl-bis(2-chloroethyl)amine—Hydrochloride : Hygroscopic colorless crystals (Me_2CO), m.p. 178~179°. Yield, 30%. *Anal.* Calcd. for $C_8H_{18}NCl_3$: C, 40.95; H, 7.73; N, 5.96. Found : C, 41.05; H, 7.68; N, 5.98.

N-*tert*-Butyl-bis(2-chloroethyl)amine N-oxide—Picrate : m.p. 95~96°. Yield, 48%. *Anal.* Calcd. for $C_{14}H_{20}O_8N_4Cl_2$: C, 38.04; H, 4.57; N, 12.68. Found : C, 37.99; H, 4.61; N, 12.82.

Hydrochloride : m.p. 89° ((iso-Pr)$_2$O). *Anal.* Calcd. for $C_8H_{18}ONCl_3$: C, 38.34; H, 7.23. Found: C, 38.21; H, 7.10.

N-Hexyl-bis(2-chloroethyl)amine—Free base : $b.p_{7.5}$ 125~129°. Hydrochloride : m.p. 78~82° (reported[8] m.p. 82.2~82.8).

N-Dodecyl-bis(2-chloroethyl)amine—Hydrochloride did not crystallize and the insoluble free base separated instantly when dissolved in water. Picrylsulfonate : m.p. 92~93°. *Anal.* Clacd. for $C_{22}H_{36}O_9N_4Cl_2S$: C, 43.94; H, 6.01. Found : C, 43.74; H, 5.58.

10-(2-Chloroethyl)phenothiazine[9]—A mixture of 10-(2-hydroxyethyl)phenothiazine[10] (100 g.), PCl_3 (140 g.), and benzene (100 cc.) was refluxed for 8 hr., distilled *in vacuo*, and the residue was poured onto crushed ice. An oily product was extracted with benzene, which was washed with 2% NaOH, dried, and concentrated, from which yellow crystals, m.p. 96~97° (EtOH), separated.

N-[2-(10-Phenothiazinyl)ethyl]-bis(2-hydroxyethyl)amine—Diethanolamine (60 g.) was added under stirring to a mixture of 10-(2-chloroethyl)phenothiazine (83 g.), K_2CO_3 (100 g.), and EtOH (200 cc.). The whole mixture was refluxed for 2 days, filtered, concentrated, poured into water, and extracted with benzene. The benzene solution was shaken with 10% HCl and the aqueous layer was made alkaline with 40% NaOH. The oil that separated was taken up in benzene, washed with water, and dried. After removal of the solvent, the residue was distilled *in vacuo*, $b.p_{0.2}$ 132~140°. Yield, 57 g.

N-[2-(10-Phenothiazinyl)ethyl]-bis(2-chloroethyl)amine—To a benzene solution of N-2-(10-phenothiazinyl)ethyl]-bis(2-hydroxyethyl)amine (24 g.), PCl_3 (20 g.) was added under stirring and the mixture was warmed slightly. The chlorination mixture was poured onto crushed ice and the separated oil was taken up in benzene. The benzene extract was again extracted with conc. HCl and the acid layer was chilled, from which the hydrochloride separated, m.p. 96~98° (dichloroethane). *Anal.* Calcd.

6) T. Yoshida : Paper read at the 15th General Assembly of Japan Medical Congress, Tokyo, April, 1959.
7) M. Ishidate, T. Yoshida, Y. Sakurai, *et al.* : Gann, **45**, 484(1954).
8) E. Wilson, M. Tishler : J. Am. Chem. Soc., **73**, 3635(1951).
9) H. Gilman, D. Shirley : *Ibid.*, **66**, 890(1944).
10) R. Dahlbom : Acta Chem. Scand., **6**, 310(1952).

for $C_{18}H_{21}N_2Cl_3S$: C, 53.65; H, 5.24. Found : C, 53.26; H, 5.37.

9-Aminocamphor—A mixture of 9-oxocamphor oxime (71 g.), EtOH (300 cc.), and liquid ammonia (100 g.), added with Raney Ni (10 g.), was shaken in H_2 at 82 atm. for 3 hr. The temperature was raised to 130°. After removal of the catalyst, the reaction mixture was acidified and evaporated. The residue was dissolved in water and extracted once with Et_2O. The aqueous layer was made alkaline and extracted again with Et_2O. The ether layer was dried and evaporated to dryness. Free base, m.p. 172°. Yield, 70 g.
Hydrochloride : m.p. 334° (decomp.). *Anal.* Calcd. for $C_{10}H_{18}ONCl$: N, 6.87. Found : N, 6.54.
Toluenesulfonamide : m.p. 182° (50% EtOH). *Anal.* Calcd. for $C_{17}H_{23}O_3NS$: C, 63.52; H, 7.21; N, 4.36. Found : C, 63.52; H, 7.28; N, 4.16.

9-Bis(2-hydroxyethyl)aminocamphor—Ethylene oxide (19.4 g.) was passed through a solution of 9-aminocamphor (33.4 g.) in MeOH at room temperature under stirring. Hydrochloride : Hygroscopic crystals, m.p. 223°. Picrate : m.p. 116~119°.

9-Bis(2-chloroethyl)aminocamphor—Hydrochloride : m.p. 218~220° (decomp.) (EtOH). *Anal.* Calcd. for $C_{14}H_{24}ONCl_3$: C, 51.15; H, 7.36; N, 4.26. Found : C, 51.40; H, 7.37; N, 4.40. $[\alpha]_D^{23}$ −60.6 (l=1, c=1.58, H_2O).

9-Bis(2-chloroethyl)aminocamphor N-Oxide—Hydrochloride : m.p. 179~180° (decomp.). *Anal.* Calcd. for $C_{14}H_{24}O_2NCl_3$: C, 48.78; H, 7.02; N, 4.06. Found : C, 48.78; H, 7.34; N, 3.97.

N-(2-Methoxyethyl)-bis(2-chloroethyl)amine—Hydrochloride, m.p. 130° (AcOEt). *Anal.* Calcd. for $C_7H_{16}ONCl_3$: C, 35.53; H, 6.82. Found : C, 35.44; H, 6.38.

N-(2-Methoxyethyl)-bis(2-chloroethyl)amine N-Oxide—Hydrochloride : m.p. 109~111° (AcOEt). *Anal.* Calcd. for $C_7H_{16}O_2NCl_3$: C, 33.28; H, 6.39. Found : C, 33.55; H, 6.86. Picrate : m.p. 89~90°. *Anal.* Calcd. for $C_{13}H_{18}O_9N_4Cl_3$: C, 35.15; H, 4.08. Found : C, 35.22; H, 3.95.

N-(2-Butoxyethyl)-bis(2-chloroethyl)amine—Hydrochloride : m.p. 126~127° (Me_2CO). *Anal.* Calcd. for $C_{10}H_{22}ONCl_3$: C, 43.08; H, 7.96. Found : C, 43.37; H, 8.16.

N-(2-Butoxyethyl)-bis(2-chloroethyl)amine N-Oxide—Hydrochloride : m.p. 75~76°. *Anal.* Calcd. for $C_{10}H_{22}O_2NCl_3$: C, 40.74; H, 7.53. Found : C, 40.57; H, 7.47. Picrate : m.p. 81~82°. *Anal.* Calcd. for $C_{15}H_{24}O_2N_4Cl_3$: C, 39.41; H, 4.96. Found : C, 39.65; H, 4.71.

N-(2-Phenoxyethyl)-bis(2-chloroethyl)amine—Hyrochloride : m.p. 150~151° (AcOEt). Picrate : m.p. 117~118° (MeOH). *Anal.* Calcd. for $C_{18}H_{20}O_8N_4Cl_2$: C, 44.00; H, 4.10. Found : C, 44.21; H, 4.08.

N-(2-Phenoxyethyl)-bis(2-chloroethyl)amine N-Oxide—Hydrochloride : m.p. 173~174° (decomp.) (MeOH). Picrate : m.p. 126~127° (Me_2CO). *Anal.* Calcd. for $C_{18}H_{20}O_9N_4Cl_2$: 42.62; H, 3.97. Found : C, 42.92; H, 3.77.

Bis(2-chloroethyl)aminoacetaldehyde—Bis(2-chloroethyl)aminoacetaldehyde dimethyl acetal was heated with 20% HCl at 80° for 5 hr. The solution was evaporated to dryness *in vacuo*, the residue was dissolved in water, added dropwise into an aqueous solution of picric acid under stirring.
Picrate: yellow crystals, m.p. 131°. *Anal.* Calcd. for $C_{12}H_{14}O_8N_4Cl_2$: C, 35.03; H, 3.39; N, 13.59. Found : C, 34.85; H, 3.46; N, 14.17.

Bis(2-chloroethyl)aminoacetaldehyde Dimethyl Acetal—Hydrochloride : m.p. 129~130°. *Anal.* Calcd. for $C_8H_{18}O_2NCl_3$: C, 35.53; H, 6.82. Found : C, 35.44; H, 6.38.

Bis(2-chloroethyl)aminoacetaldehyde Dimethyl Acetal N-Oxide—Hydrochloride : m.p. 110°.
Picrate : m.p. 89~90°. *Anal.* Calcd. for $C_{14}H_{20}O_{10}N_4Cl_2$: C, 35.15; H, 4.08. Found : C, 35.22; H, 3.95.

Bis(2-chloroethyl)aminoacetaldehyde Diethyl Acetal—Hydrochloride : m.p. 132~133°. *Anal.* Calcd. for $C_{10}H_{22}O_2NCl_3$: C, 40.76; H, 7.55; N, 4.88. Found : C, 40.60; H, 7.88; N, 5.24.

Bis(2-chloroethyl)aminoacetaldehyde Dibutyl Acetal—Free base : Colorless oil, $b.p_3$ 111~113°. Hydrochloride : m.p. 121~122° (MeCOPr). *Anal.* Calcd. for $C_{14}H_{30}O_2NCl_3$: C, 47.93; H, 8.62. Found: C, 47.75; H, 8.90.

4-Bis(2-chloroethyl)amino-2-butanone—A mixture of bis(2-chloroethyl)amine hydrochloride (41 g.), 30% HCHO (23 cc.), and Me_2CO (95 cc.) was slightly warmed under stirring, maintained at 40~45° for 1.5 hr. with warming, if necessary, and then at room temperature over night. The reaction mixture was evaporated to dryness *in vacuo* and the oily residue was dissolved in a small quantity of water. To this solution, a MeOH solution of picric acid was added and a yellow crystalline picrate, m.p. 113~115°, was obtained. *Anal.* Calcd. for $C_{14}H_{18}O_8N_4Cl_2$: C, 38.19; H, 3.99. Found : C, 38.10; H, 4.11.

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Summary

Twenty new derivatives of nitrogen mustard, including six N-oxides, were prepared and tested for their *in vivo* and *in vitro* antitumor activity against the Yoshida sarcoma.

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19. Ryuichi Kimura, Takahiro Yabuuchi, and Yasutaka Tamura : Studies on Thiophene Derivatives. IV.[1] Syntheses of 2-Aminoethyl 3,3-Diaryl-3-hydroxypropanoates and 3,3-Diaryl-2-propenoates.

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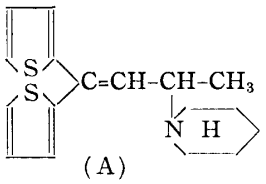
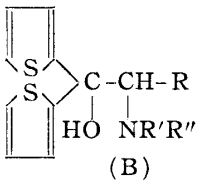
It is known that some thiophene derivatives have an interesting pharmacological action. For example, 3-piperidino-1,1-di(2-thienyl)-1-butene (A) has a more potent antitussive action than morphine or Methadone.

In previous papers[1,2] of this series, it has been shown that the antitussive effect of the dextro form obtained by optical resolution of (A) compound was twice as strong as the racemic form and also several times stronger than codeine in clinical application.

In a later work,[3] many derivatives of 2-amino-1,1-(2-thienyl)alkanol (B) having structures similar to both ephedrine and (A) compound were synthesized in order to obtain new active antitussive agents in this field.



(A)



(B)

As the inhibitor of parasympathetic system generally relieves convulsion of the bronchus, some of them exert an antitussive action. For example, 2'-diethylaminoethyl 1-phenylcyclopentane-1-carboxylate (C) and 2-aminoalkyl 3,3-diphenyl 2-propenoic acids (D) have antitussive effect. Therefore, it seemed of interest to synthesize some derivatives of 2-aminoethyl 3,3-diaryl-2-propenoates (F), in which R_1 represents thienyl group, R_2 the thienyl group or phenyl group, and NR'R'' the aliphatic amine or cycloamine. None of these compounds seem to have been prepared.

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1) Part III : This Bulletin, **7**, 175(1959).
2) R. Kimura, T. Yabuuchi : *Ibid.*, **7**, 171(1959).
3) *Idem.* : *Ibid.*, **6**, 159(1958).