

m.p. 203~205.5°. $[\alpha]_D^{20} +12^\circ$ ($c=1.03$, CHCl_3), IR $\nu_{\text{max}}^{\text{CS}_2}$ cm^{-1} : 3640 (-OH), 1717 (3-oxo). *Anal.* Calcd. for $\text{C}_{20}\text{H}_{32}\text{O}_2$: C, 78.89; H, 10.59. Found: C, 78.62; H, 10.48.

17 β -Hydroxy-1 α -methylandrost-4-en-3-one (VIb)—17 β -Hydroxy-1 α -methyl-5 α -androstan-3-one (IVb) (205 mg.) was brominated and dehydrobrominated as described above. The product was chromatographed on alumina and the material eluted with benzene-Et₂O was recrystallized from Me₂CO-hexane to give 17 β -hydroxy-1 α -methylandrost-4-en-3-one (VIb), m.p. 183~187.5°. Yield, 93 mg. Further recrystallization gave white needles, m.p. 188~190°, $[\alpha]_D^{17} +144^\circ$ ($c=0.624$, CHCl_3). UV: $\lambda_{\text{max}}^{\text{EtOH}}$ 241 m μ ($\log \epsilon$ 4.13). IR $\nu_{\text{max}}^{\text{CS}_2}$ cm^{-1} : 3610 (-OH), 1672, 1615 (4-en-3-oxo). *Anal.* Calcd. for $\text{C}_{20}\text{H}_{30}\text{O}_2$: C, 79.42; H, 10.00. Found: C, 79.51; H, 9.96.

The author is very grateful to Dr. M. Chuman, President of Tsurumi Research Laboratory of Chemistry, Dr. S. Niinobe, Director of Research Laboratory of this company, and Mr. M. Sawai for their valuable advices, and to Dr. E. Yamaguchi, President of this company, and Dr. F. Ueno, Director of Manufacturing Section of this company, for their encouragement throughout this work. The author is also indebted to Mr. K. Tsuneda for his technical help.

Summary

The Grignard reaction of 5 α -cholest-1-en-3-one (IIIa) in the presence of cuprous chloride gave 1 α -methyl-5 α -cholestan-3-one (IVa). (IVa) was brominated and dehydrobrominated to give 1 α -methylcholest-4-en-3-one (VIa). (VIa) was hydrogenated over palladium-charcoal to give 1 α -methyl-5 β -cholestan-3-one (VII). Discussions were made on configuration of C-1 methyl group.

Similarly, 17 β -hydroxy-5 α -androst-1-en-3-one (IIIb) was transformed into (IVb) and (VIb).

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63. Kenichi Sawatari: Studies on Carcinostatic Substances. XXXIX.*¹

Antitumor Effect of Derivatives of Nitrogen Mustard
containing only one 2-Chloroethyl Group.

(Yoshitomi Pharmaceutical Industries, Ltd.*²)

In the preceding paper of this series,¹⁾ it was reported that N-(2-chloroethyl)-N-methyl-3-chloropropylamine unexpectedly exhibited a strong antitumor effect on Yoshida sarcoma and several strains of rat ascites hepatomas.

From these observations, it was deduced that 3-chloropropyl group of this compound might also play a role in biological alkylating reaction on the cell constituents of a tumor, in spite of its high inertness in reactivity estimated from chemical reactions in an aqueous solution *in vitro*.

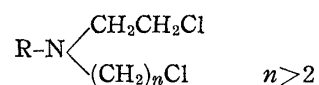
The fact seems to show that even a 2-chloroalkyl group, which does not transform into any active intermediate like aziridinium ion of 2-chloroethyl group of the amine, is able to manifest alkylation activity *in vivo*, if the remaining one functional group of the compound is active enough as a 2-chloroethyl group.

*¹ This paper constitutes a part of series entitled "Studies on Carcinostatic Substances" by M. Ishidate and Y. Sakurai. Part XXXVIII: This Bulletin, 9, 996 (1961).

*² Yoshitomi-machi, Chikujo-gun, Fukuoka-ken (猿渡健市).

1) K. Sawatari: This Bulletin, 9, 996 (1961).

To investigate antitumor activity of hypothetical bifunctional alkylating agents having only one 2-chloroethyl group, a series of compounds derived from the following formula were newly prepared.



The first group of compounds is demonstrated in Table I, in all of which R remains the same methyl and only n differs. The methods of preparation are shown in Chart 1.

TABLE I. $\text{CH}_3\text{-N} \begin{cases} (\text{CH}_2)_n\text{Cl} \\ (\text{CH}_2)_m\text{Cl} \end{cases}$

Compd. No.	n	m	LD ₅₀ ^(a) (mg./kg.)	MTD ^(e) (mg./kg.)	MED ^(f) (mg./kg.)	MEC ^(g) (m. M)
557 ^(a)	3	2	30	10	0.5	10 ⁻²
687 ^(b)	4	2	75	50	1	10 ⁻²
721 ^(c)	5	2	175	100	10	2.5 × 10 ⁻²
704 ^(b)	6	2	75	50	1	10 ⁻²
698 ^(a)	3	3	175	100	100	(-)
759 ^(c)	5	5	175	100	Inactive	
738 ^(c)	6	6	175	100	"	

a) Picrate

b) Hydrochloride

c) Picrylsulfonate

d) Rat, i. p.

e) Maximum tolerance dose on rat, i. p.

f) Minimum effective dose on Yoshida sarcoma, i. p. (M. Ishidate, *et al.* : Gann, 44, 342 (1953)).

g) Minimum effective concentration on *in vitro*-cultured Yoshida sarcoma. (M. Ishidate, *et al.* : This Bulletin, 7, 873 (1959)).

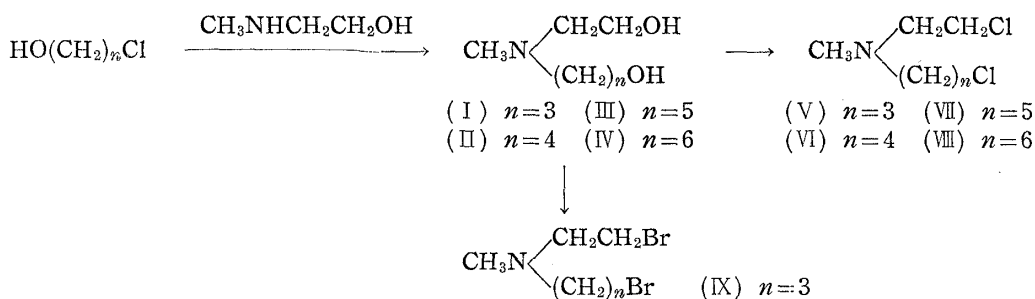


Chart 1.

The results are very striking, because all these compounds have a marked antitumor effect on Yoshida sarcoma, whereas they all exhibited only monofunctional alkylating reactivity in the *in vitro* chemical reactions, *viz.* Cl⁻ liberation and thiosulfate uptake in a neutral aqueous solution, as demonstrated in Table II.

TABLE II. Cl⁻ Liberation and Thiosulfate Uptake of $\text{CH}_3\text{-N} \begin{cases} (\text{CH}_2)_n\text{Cl} \\ (\text{CH}_2)_m\text{Cl} \end{cases}$ in NaHCO₃ Buffer Solution at 37°

Compd. No.	n	m	Medium	Cl ⁻ Liberation (molar equiv.) (min.)					Thiosulfate uptake (molar equiv.) (min.)			
				10	20	30	60	120	10	20	30	60
557	3	2	H ₂ O	0.91	0.91	0.91	0.93		0.86	0.93	0.93	0.93
687	4	2	H ₂ O	0.93	0.93	0.93	0.97	0.97	0.05	0.07	0.07	0.08
721	5	2	35% Me ₂ CO	1.02		1.05	1.10	1.20	0.72	0.81	0.81	0.81
704	6	2	40% MeOH	0.89	0.90	0.93	0.93	0.93	0.89	0.90	0.95	0.97
738	6	6	H ₂ O	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Among these compounds, the strong antitumor effect and large chemotherapeutic indices of the two compounds, No. 687 and No. 704, are particularly notable. Especially the effect of the latter on Yoshida sarcoma seems to be of the same order as that of Nmethyl-2,2'-dichlorodiethylamine N-oxide, notwithstanding the fact that the reactivity of chlorine in 6-chlorohexyl group must be no more than that of the ordinary chloride alkyls.

The most outstanding is the fact that No. 689 liberates very rapidly, nearly within 10 minutes, one molar equivalent of chlorine ion in a bicarbonate buffer solution at 37° but scarcely consumes thiosulfate even in the presence of an excess of thiosulfate in the reaction solution. However, the following experiment may reveal the mechanism of these reactions.

An aqueous mixture of No. 687 and sodium thiosulfate was buffered with sodium bicarbonate and incubated for 10 minutes at 37°, and a solution of picric acid was poured at once into this solution. A picrate, melting at 177~178°, precipitated instantly in nearly quantitative yield, which was identified as N-(2-chloroethyl)-N-methylpyrrolidinium picrate.

In short, a leading reaction of No. 687 in a solution is the formation of a pyrrolidinium ring derivative which no longer has antitumor activity. Nevertheless, No. 687 must behave against tumors as a biological bifunctional alkylating agent, because it was proved to have a strong effect on tumors *in vivo*.

The fact may suggest therefore that there are some acceptors in the cell more sensitive to alkylating agents than mercapto group of thiosulfate and they attract competitively some part of functional group of No. 687 toward themselves.

The compounds having a general formula, $\text{CH}_3\text{-N}[(\text{CH}_2)_n\text{Cl}]_2$ ($n > 2$), have of course no reactivity *in vitro* and also no tumor inhibiting effect, as seen in Table I.

The second group of compounds having various substituents at R are demonstrated in Table III, preparation of which is shown in Chart 2.

TABLE III. $\text{R-N} \begin{cases} \text{CH}_2\text{CH}_2\text{Cl} \\ (\text{CH}_2)_n\text{Cl} \end{cases}$

Compd. No.	R	n	LD ₅₀ (mg./kg.)	MTD (mg./kg.)	MED (mg./kg.)	MEC (m. M)
690 ^{a)}	$\text{ClCH}_2\text{CH}_2\text{CH}_2\text{-}$	3	30	10	0.5	2.5×10^{-3}
694 ^{b)}	$\text{C}_2\text{H}_5\text{OC}_2\text{H}_4\text{-}$	3	3	1	0.5	1×10^{-2}
695 ^{b)}	$\text{C}_6\text{H}_5\text{CH}_2\text{-}$	3	75	50	5	5×10^{-3}
696 ^{b)}	$(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{-}$	3	75	50	5	5×10^{-3}
722 ^{b)}	$\text{C}_2\text{H}_5\text{OC}_2\text{H}_4\text{-}$	4	75	50	Inactive	
729 ^{c)}	$\text{C}_6\text{H}_5\text{CH}_2\text{-}$	4	375	250	"	
730 ^{c)}	$n\text{-C}_4\text{H}_9\text{-}$	4	375	250	"	
731 ^{c)}	$(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{-}$	4	175	100	"	
735 ^{a)}	$\text{H}_2\text{NOCCH}_2\text{-}$	4	175	100	50	(-)
742 ^{c)}	$\text{C}_6\text{H}_5\text{CH}_2\text{-}$	6	175	100	10	
743 ^{c)}	$\text{HOOCCH}_2\text{-}$	4	175	100	Inactive	
744 ^{c)}	$\text{C}_2\text{H}_5\text{OOCCH}_2\text{-}$	4	175	100	"	
748 ^{c)}	$\text{H}_2\text{NOCCH}_2\text{-}$	3	3	1	0.5	2.5×10^{-3}
749 ^{c)}	$\text{C}_2\text{H}_5\text{OOCCH}_2\text{-}$	3	7.5	5	0.5	1×10^{-2}
758 ^{c)}	$\text{HOOCCH}_2\text{-}$	3	30	10	0.5	1×10^{-1}
760 ^{c)}	$\text{C}_6\text{H}_5\text{CH}_2\text{-}$	5	175	100	10	(-)

a) Picrate

b) Hydrochloride

c) Picrylsulfonate

There was found no exception as to the monofunctional alkylating reactivity of these compounds in the *in vitro* reactions but, also in these cases, the compounds belonging to N-alkyl-N-(2-chloroethyl)-3-chloropropylamine series were all proved to have a marked effect in inhibiting tumor growth. However, in a series of N-alkyl-N-(2-chloroethyl)-

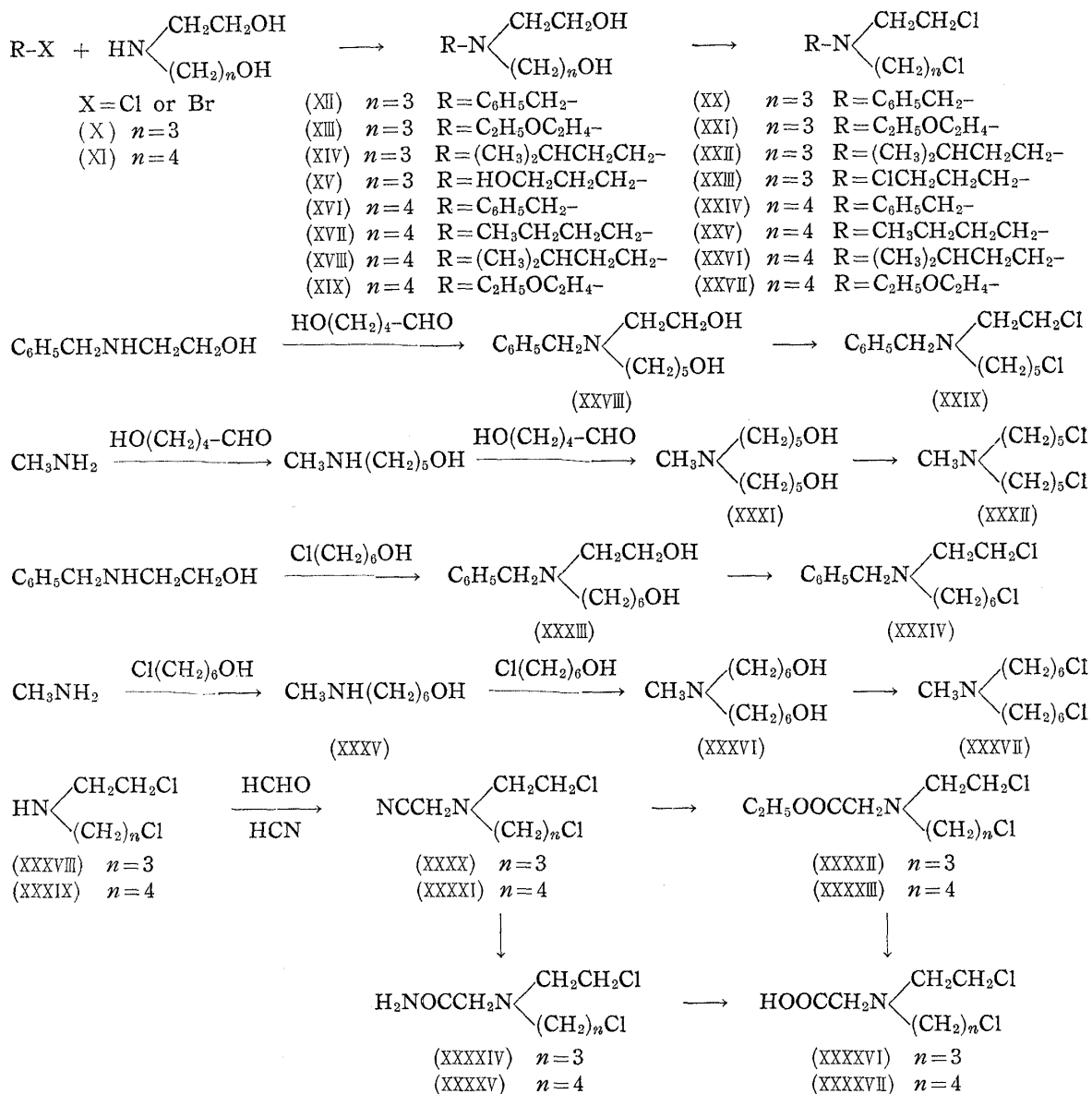


Chart 2.

4-chlorobutylamine shown in Table III, only the carbamylmethyl derivative (No. 735) alone inhibited tumor growth. Ineffectiveness of the remaining derivatives may be due to their strong tendency to undergo intramolecular cyclization to pyrrolidinium forms than that of No. 687 or No. 735, in which R is methyl or carbamylmethyl group. That methyl substitute behaves in a particular way in either chemical or biological reactions different from other alkyl groups has been often experienced in some field of chemistry and pharmacology.

In Table IV are shown some related but miscellaneous types of compounds.

Among these compounds prepared in this work, two compounds, No. 687 and No. 695, are distinguished for their large chemotherapeutic index ($\text{LD}_{50}/\text{MED}^{*3}$) and also for having wider antitumor spectrum than that of N-methyl-2,2'-dichlorodiethylamine N-oxide against rat ascites hepatomas, about which the experimental results will be published elsewhere in the near future.

*3 MED: Minimum effective dose on Yoshida sarcoma.

TABLE IV.

Compd. No.	Compound	LD ₅₀ (mg./kg.)	MTD (mg./kg.)	MED (mg./kg.)	MEC (mg./kg.)
697	$\begin{array}{c} \text{CH}_2\text{---O} \\ \\ \text{CH}_2\text{---CH}_2 \end{array} \text{N}^+ \begin{array}{l} \text{CH}_2\text{CH}_2\text{Cl} \\ \text{CH}_2\text{CH}_2\text{CH}_2\text{Cl} \end{array} \cdot \text{Picrylsulfonate}$	175	100	5	(—)
701	$\begin{array}{c} \text{CH}_2\text{---CH}_2 \\ \\ \text{CH}_2\text{---CH}_2 \end{array} \text{N}^+ \begin{array}{l} \text{CH}_3 \\ \text{CH}_2\text{CH}_2\text{Cl} \end{array} \cdot \text{Picrate}$	75	50	Inactive	
703	$\begin{array}{c} \text{CH}_2\text{---CH}_2 \\ \\ \text{CH}_2\text{---CH}_2 \end{array} \text{N}^+ \text{---CH}_2\text{CH}_2\text{Cl} \cdot \text{Hydrochloride}$	375	250	"	
707	$\text{CH}_3\text{---N} \begin{array}{l} \text{CH}_2\text{CH}_2\text{Br} \\ \text{CH}_2\text{CH}_2\text{CH}_2\text{Br} \end{array} \cdot \text{Picrate}$	3	1	0.5	6 × 10 ⁻⁴
733	$\begin{array}{c} \text{CH}_3\text{---N} \\ \\ \text{O} \end{array} \begin{array}{l} \text{CH}_2\text{CH}_2\text{Cl} \\ \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{Cl} \end{array} \cdot \text{Picrylsulfonate}$	375	250	250	(—)

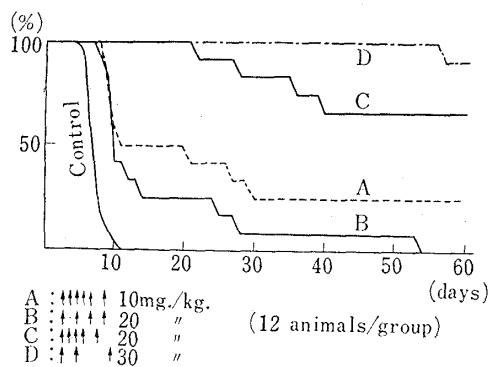


Fig. 1. Percentage Survival Diagram of Yoshida Sarcoma Rats Treated with Compound No. 687

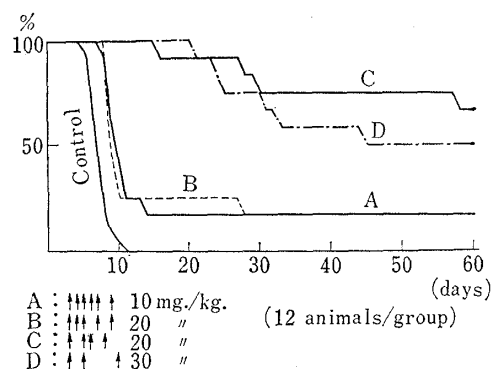


Fig. 2. Percentage Survival Diagram of Yoshida Sarcoma Rats Treated with Compound No. 695

The percentage survival diagrams obtained by repeated injection of each of these two compounds in rats bearing Yoshida sarcoma are shown in Figs. 1 and 2.

It is worthy to note that the result from No. 687 (Fig. 2.) was far better than expected, considering its inertness in alkylating reactivity *in vitro*.

An attempt was made to convert these active agents to masked forms by oxidation to their N-oxides. Actually, No. 687 gave its N-oxide by the usual process with peracid, but tertiary amines having 3-chloropropyl group gave no primary N-oxides but N,N-disubstituted isoxazolidinium chlorides as was described by Ishidate, Sakurai, *et al.*²⁾

Of the compounds demonstrated in Table IV, No. 733 was only slightly effective and No. 697 effective, but even the efficacy of No. 697 dose not match that of N-methyl-2,2'-dichlorodiethylamine N-oxide or No. 687. The poor efficacy of No. 697 may be due to the too slow reduction of its ring to 3-[(2-chloroethyl)(3-chloropropyl)amino]-1-propanol *in vivo*.

As a conclusion, it can be said that even the derivatives of nitrogen mustard having only one 2-chloroethyl group can be sufficiently effective in inhibiting tumor growth, if they have one more chloride alkyl group, reactivity of which is not necessary to be greater than that of ordinary chloride alkyls such as ethyl chloride.

This new finding seems to indicate that there may be a certain strong nucleophilic site of reaction in cells which can attract selectively to some extent such a type of

2) M. Ishidate, Y. Sakurai, *et al.*: This Bulletin, 9, 485 (1961).

bifunctional electrophilic reagent. The fact is believed to add something new to the comprehension of the mode of action of alkylating agents having an antitumor activity.

Experimental

3-[(2-Ethoxyethyl)(2-hydroxyethyl)amino]-1-propanol (XIII)—A mixture of ethoxyethylbromide (50 g.) and 3-[(2-hydroxyethyl)amino]-1-propanol (60 g.) was heated at 80° for 1 hr. After basification with 30% KOH, the precipitated KBr was removed. The filtrate was extracted with benzene, the extract was dried over anhyd. K_2CO_3 , and fractionated *in vacuo*. b.p.₃₀ 185~190°. Yield, 80%.

3-[(2-Hydroxyethyl)(3-methylbutyl)amino]-1-propanol (XIV)—It was obtained from isoamyl bromide (75 g.) and 3-[(2-hydroxyethyl)amino]-1-propanol (60 g.) by the same procedure as above. b.p.₃₀ 183~186°. Yield, 60%.

3-[Benzyl(2-hydroxyethyl)amino]-1-propanol (XII)—A mixture of BzCl (65 g.) and 3-[(2-hydroxyethyl)amino]-1-propanol (60 g.) was heated for 1 hr. at 70°. b.p.₄ 170~175°.

3-[(2-Hydroxyethyl)(3-hydroxypropyl)amino]-1-propanol (XV)—A mixture of trimethylenechlorohydrin (48 g.), 3-[(2-hydroxyethyl)amino]-1-propanol (58 g.), and PrOH (100 cc.) was refluxed for 15 hr. After removal of the inorganic salt by filtration, PrOH was distilled off and the residue was fractionated. b.p.₄ 187~190°.

4-[(2-Hydroxyethyl)amino]-1-butanol (XI)—Tetramethylenechlorohydrin (109 g.) was added dropwise during 3 hr. to a solution of 2-aminoethanol (61 g.) in MeOH (150 cc.) under reflux. The mixture was stirred for 2 hr. more at the same temperature and then neutralized with 30% KOH. After removal of KCl, the filtrate was fractionated *in vacuo*. b.p.₃ 140~143°.

4-[(2-Hydroxyethyl)methylamino]-1-butanol (II)—It was obtained from tetramethylenechlorohydrin (60 g.) and 2-methylaminoethanol (40 g.) by the same procedure as for (XI). b.p.₃ 122~126°.

4-[(2-Hydroxyethyl)ethoxyethylamino]-1-butanol (XIX)—Ethoxyethyl bromide (46 g.) was added dropwise into a solution of 4-[(2-hydroxyethyl)amino]-1-butanol (40 g.) in MeOH (100 cc.). The mixture was then heated for 3 hr. at 70° under a reflux condenser. b.p.₃ 135~141°.

4-[Benzyl-(2-hydroxyethyl)amino]-1-butanol (XVI)—A mixture of BzCl (64 g.), 4-[(2-hydroxyethyl)amino]-1-butanol (67 g.), anhyd. K_2CO_3 (70 g.), and EtOH (100 cc.) was refluxed for 15 hr. After filtration, the filtrate was fractionated *in vacuo*. b.p.₄ 170~175°. Yield, 83%.

4-[(2-Hydroxyethyl)(3-methylbutyl)amino]-1-butanol (XVIII)—Isoamylbromide (50 g.) and 30% KOH (70 cc.) were added dropwise simultaneously from two different dropping funnels into a solution of 4-[(2-hydroxyethyl)amino]-1-butanol (45 g.) in water (100 cc.) over 2 hr. at 70°. The mixture was stirred for 2 hr. more at 80°. After filtration, it was extracted with benzene, the extract was dried over anhyd. K_2CO_3 , and fractionated *in vacuo*. b.p.₃ 135~142°.

4-[(2-Hydroxyethyl)butylamino]-1-butanol (XVII)—It was obtained from butyl bromide (50 g.), 30% KOH (70 cc.), and a solution of 4-[(2-hydroxyethyl)amino]-1-butanol (50 g.) in water (100 cc.) by the same procedure as for (XVIII). b.p.₃ 130~137°.

5-[(2-Hydroxyethyl)methylamino]-1-pentanol (III)—A mixture of N-2-hydroxyethyl-methylamine (27.8 g.), pentamethylenechlorohydrin (45 g.), K_2CO_3 (55 g.), and EtOH (100 cc.) was boiled for 10 hr. with stirring. EtOH was removed and the filtrate was fractionated *in vacuo*. b.p.₄ 135~148°.

5-[Benzyl(2-hydroxyethyl)amino]-1-pentanol (XXVIII)—5-Hydroxypentanal (21 g.) was gradually added into 2-(benzylamino)ethanol (30 g.), previously cooled in a freezing mixture, and then 80% HCOOH (16 g.) was added into this mixture. After the evolution of heat abated, the reaction mixture was again heated at 90~95° for 3 hr. and fractionated. b.p.₃ 185~193°. Yield, 70%.

5-(Methylamino)-1-pentanol (XXX)—5-Hydroxypentanal (60 g.), methylamine (45 g.) dissolved in MeOH (300 cc.), and Raney Ni (10 g.) were placed in an autoclave, which was shaken with H_2 (15 atm.) at 110° for 5 hr. The catalyst was then removed and the filtrate was fractionated *in vacuo*. b.p.₃ 93~95°.

N-Methyl-5,5'-dihydroxydipentylamine (XXXI)—It was obtained by the reaction of 5-hydroxypentanal (54 g.) and N-5-hydroxypentylmethylamine (60 g.) under hydrogenation as described for (XXX). b.p.₃ 170~175°.

6-[(2-Hydroxyethyl)methylamino]-1-hexanol (IV)—Hexamethylenechlorohydrin (50 g.) was added slowly and dropwise into a solution of 2-(methylamino)ethanol (27.8 g.) in MeOH (100 cc.) at 80°. The mixture was boiled under stirring for 4 hr., basified with 30% KOH, and extracted with benzene. The extract was dried over anhyd. K_2CO_3 and fractionated *in vacuo*. b.p.₄ 143~145°.

6-(Methylamino)-1-hexanol (XXXV)—Hexamethylenechlorohydrin (30 g.) was added dropwise into a solution of methylamine (30 g.) in water (70 cc.) and the mixture was warmed at 50° for 20 hr. It was added with 30% KOH and after removal of the separated KCl, water was distilled off and the residue was fractionated *in vacuo*. b.p.₃ 120~125°.

N-Methyl-6,6'-dihydroxydihexylamine (XXXVI)—A mixture of hexamethylenechlorohydrin (20 g.), 6-(methylamino)-1-hexanol (20 g.), K_2CO_3 (50 g.), and MeOH (100 cc.) was warmed at 50~60° for 30 hr.

The separated KCl was removed and MeOH was distilled off. The residue was fractionated *in vacuo*. b.p. 168~175°.

6-[Benzyl(2-hydroxyethyl)amino]-1-hexanol (XXXIII)—It was obtained from hexamethylenechlorohydrin (30 g.) and 2-benzylaminoethanol (20 g.) by the same procedure as for (XXXVI). b.p. 185~195°.

1-Pyrrolidinethanol—The Ethyleneoxide (20 g.) was passed into an ice-cooled solution of pyrrolidine (25 g.) in water (100 cc.). After being kept over night at room temperature, the reaction mixture was fractionated. b.p. 120~135°. Yield, 25 g.

N-Methyl-N-2-hydroxyethylpyrrolidinium Picrate—A mixture of N-methylpyrrolidine (20 g.) and ethylenechlorohydrin (40 g.) was heated at 80~90° for 10 hr. The excess of ethylenechlorohydrin was completely removed by distillation. N-Methyl-N-2-hydroxyethylpyrrolidinium chloride was obtained as a hygroscopic white crystalline solid. Picrate, m.p. 223~225° (from EtOH). *Anal.* Calcd. for $C_{13}H_{19}O_8N_4$: C, 43.45; H, 5.33; N, 15.60. Found: C, 43.82; H, 4.74; N, 15.39.

General Procedure for Chlorination of the Hydroxy-amines— $SOCl_2$ (0.21 mole) was added dropwise into a mixture of the hydroxyamine (0.1 mole) in benzene or $CHCl_3$ (200 cc.) under cooling in an ice-water bath. The mixture was then carefully warmed to 80° during several hrs. until evolution of HCl gas ceased. The solvent and the excess of $SOCl_2$ were removed *in vacuo* and the residue was recrystallized as usual from EtOH. When the hydrochloride was not obtained as crystals, it was converted into a picrate or picrylsulfonate. For purification, in some cases, the crude hydrochloride was dissolved in water, basified with alkali, and extracted with benzene. After removal of the solvent *in vacuo*, the free base was fractionated *in vacuo* and converted again to a hydrochloride or a picrate.

N-(2-Chloroethyl)-N-(2-ethoxyethyl)-3-chloropropylamine (No. 694) (XXI)—Hydrochloride, m.p. 97~98° (from AcOEt). *Anal.* Calcd. for $C_9H_{20}ONCl_3$: C, 40.84; H, 7.62; N, 5.29. Found: C, 40.92; H, 7.42; N, 5.22.

N-Benzyl-N-(2-chloroethyl)-3-chloropropylamine (No. 695) (XX)—Hydrochloride, m.p. 99~100° (from EtOH). *Anal.* Calcd. for $C_{12}H_{18}NCl_3$: C, 51.00; H, 6.42; N, 4.95. Found: C, 51.29; H, 6.40; N, 5.13.

N-(2-Chloroethyl)-N-(3-methylbutyl)-3-chloropropylamine (No. 696) (XXII)—Hydrochloride, m.p. 85~87° (from EtOH). *Anal.* Calcd. for $C_{10}H_{22}NCl_3$: C, 45.73; H, 8.44; N, 5.33. Found: C, 45.83; H, 8.24; N, 5.52.

N-(2-Chloroethyl)-3,3'-dichlorodipropylamine (No. 690) (XXIII)—Picrate, m.p. 78~79° (from EtOH). *Anal.* Calcd. for $C_{14}H_{19}O_7N_4Cl_3$: C, 36.42; H, 4.15; N, 12.11. Found: C, 36.67; H, 4.13; N, 12.10.

N-(2-Chloroethyl)-N-methyl-4-chlorobutylamine (No. 687) (VI)—Hydrochloride, m.p. 87~88° (from AcOEt). *Anal.* Calcd. for $C_7H_{16}NCl_3$: C, 38.11; H, 7.30; N, 6.35; Cl, 48.22. Found: C, 37.70; H, 7.23; N, 5.98; Cl, 48.15.

N-(2-Chloroethyl)-N-(2-ethoxyethyl)-4-chlorobutylamine (No. 722) (XXVII)—Hydrochloride, m.p. 92~93° (from EtOH-AcOEt). *Anal.* Calcd. for $C_{10}H_{22}ONCl_3$: C, 43.11, H, 7.96; N, 5.03. Found: C, 43.47; H, 8.25; N, 5.20.

N-(2-Chloroethyl)-4-chlorodibutylamine (No. 730) (XXV)—Picrylsulfonate, m.p. 163~164° (from EtOH). *Anal.* Calcd. for $C_{16}H_{24}O_9N_4Cl_2S$: C, 37.00; H, 4.66; N, 10.79. Found: C, 37.00; H, 4.80; N, 10.71.

N-(2-Chloroethyl)-N-(3-methylbutyl)-4-chlorobutylamine (No. 731) (XXVI)—Picrylsulfonate, m.p. 166~167° (from EtOH). *Anal.* Calcd. for $C_{17}H_{26}O_9N_4Cl_2S$: C, 38.28; H, 4.91; N, 10.50. Found: C, 38.47; H, 4.71; N, 10.53.

N-(2-Chloroethyl)-N-(4-chlorobutyl)benzylamine (No. 729) (XXIV)—Picrylsulfonate. m.p. 166~167° (from MeOH). *Anal.* Calcd. for $C_{19}H_{22}O_9N_4Cl_2S$: C, 41.23; H, 4.01; N, 10.13. Found: C, 41.20; H, 4.14; N, 10.29.

N-(2-Chloroethyl)-N-methyl-5-chloropentylamine (No. 721) (VII)—Free base, b.p. 75~85° (under partial polymerization). Picrylsulfonate, m.p. 145~146° (from MeOH). *Anal.* Calcd. for $C_{14}H_{20}O_9N_4Cl_2S$: C, 34.23; H, 4.10; N, 11.41. Found: C, 34.21; H, 3.99; N, 11.31.

N-(2-Chloroethyl)-N-(5-chloropentyl)benzylamine (No. 760) (XXIX)—Picrylsulfonate, m.p. 128~129° (from MeOH). *Anal.* Calcd. for $C_{20}H_{24}O_9N_4Cl_2S$: C, 42.33; H, 4.34; N, 9.88. Found: C, 42.03; H, 4.10; N, 9.97.

N-Methyl-5,5'-dichlorodipentylamine (No. 759) (XXXII)—Picrylsulfonate, m.p. 119~120°. *Anal.* Calcd. for $C_{17}H_{26}O_9N_4Cl_2S$: C, 38.28; H, 5.10; N, 10.52. Found: C, 38.58; H, 4.86; N, 10.48.

N-(2-Chloroethyl)-N-methyl-6-chlorohexylamine (No. 704) (VIII)—Hydrochloride, m.p. 83~84° (from EtOH). *Anal.* Calcd. for $C_9H_{20}NCl_3$: C, 43.47; H, 8.11; N, 5.63. Found: C, 43.74; H, 8.02; N, 5.54.

N-(2-Chloroethyl)-N-(6-chlorohexyl)-benzylamine (No. 742) (XXXIV)—Picrylsulfonate, m.p. 113~114° (from EtOH). *Anal.* Calcd. for $C_{21}H_{26}O_9N_4Cl_2S$: C, 43.38; H, 4.51; N, 9.64. Found: C, 43.05; H, 4.48; N, 9.85.

N-Methyl-6,6'-dichlorodihexylamine (No. 738) (XXXVII)—Picrylsulfonate, m.p. 156~157°. *Anal.* Calcd. for $C_{19}H_{30}O_9N_4Cl_2S$: C, 40.64; H, 5.39; N, 9.98. Found: C, 40.69; H, 5.14; N, 10.56.

N-(2-Chloroethyl)-pyrrolidine (No. 703)—Hydrochloride. m.p. 162~163° (from EtOH). *Anal.* Calcd. for $C_6H_{13}NCl_2$: C, 42.36; H, 7.70; N, 8.23. Found: C, 42.07; H, 7.40; N, 8.00. Picrate, m.p. 104~105°.

N-Methyl-N-(2-chloroethyl)-pyrrolidinium Picrate (No. 701)—Picrate, m.p. 176~177° (from EtOH). *Anal.* Calcd. for $C_{13}H_{18}O_7N_4Cl$: C, 41.33; H, 4.80; N, 14.84. Found: C, 41.80; H, 4.64; N, 14.77.

N-(2-Chloroethyl)-3-chloropropylamine (XXXVIII)—Hydrochloride, m.p. 201~202° (from Me_2CO). *Anal.* Calcd. for $C_5H_{12}NCl_3$: C, 31.19; H, 6.43; N, 7.29. Found: C, 31.36; H, 6.48; N, 7.38.

N-(2-Chloroethyl)-4-chlorobutylamine (XXXIX)—Hydrochloride, m.p. 179~180° (from $AcOMe$). *Anal.* Calcd. for $C_6H_{14}NCl_3$: C, 34.88; H, 6.38; N, 6.78. Found: C, 34.76; H, 6.77; N, 6.63.

2-[(2-Chloroethyl)(3-chloropropyl)amino]-acetamide (No. 748) (XXXXIV)—A solution of NaCN (9.5 g.) in water (100 cc.) was added dropwise at 0° into a mixture of N-(2-chloroethyl)-3-chloropropylamine hydrochloride (42 g.), 38% HCOH (18 g.), and H_2O (150 cc.). The mixture was stirred for 1 hr. at the same temperature. The separated oil was extracted with Et_2O and the extract which contained crude N-(3-chloropropyl)-N-(2-chloroethyl)-acetonitril (XXXX), was dried over anhyd. K_2CO_3 . This extract was added into 98% H_2SO_4 (30 g.) with chilling in an ice-salt bath. After being kept over night at room temperature, the mixture was poured on crushed ice, neutralized with alkali and extracted with benzene. The benzene extract was added into picrylsulfonic acid solution. The picrylsulfonate was recrystallized from MeOH, m.p. 145~147°. *Anal.* Calcd. for $C_{13}H_{17}O_5N_5Cl_2S$: C, 30.94; H, 3.35; N, 13.54. Found: C, 30.74; H, 3.44; N, 13.81.

2-[(2-Chloroethyl)(4-chlorobutyl)amino]-acetamide (No. 735) (XXXXV)—It was obtained from N-(2-chloroethyl)-4-chlorobutylamine by the same procedure as for (XXXXIV). The crude picrate was dissolved in MeOH, into which water was gradually added. The picrate precipitated slowly from the solution and this purification was repeated three times. Picrate, m.p. 66~67°. *Anal.* Calcd. for $C_{14}H_{19}O_5N_5Cl_2$: C, 36.85; H, 4.20; N, 15.35. Found: C, 36.84; H, 4.22; N, 15.41.

N-(2-Chloroethyl)-N-(3-chloropropyl)-glycine Ethyl Ester (No. 749) (XXXXII)—A solution of the crude hydrochloride of No. 748 (10 g.) dissolved in EtOH and saturated with HCl, was refluxed for 2 hr. The separated NH_4Cl was removed and the filtrate was distilled off. The residue was dissolved in Me_2CO , filtered, and the filtrate was evaporated to dryness. The final residue was converted to the picrylsulfonate, m.p. 168~169° (from MeOH). *Anal.* calcd. for $C_{15}H_{20}O_{11}N_4Cl_2S$: C, 33.66; H, 3.65; N, 10.46. Found: C, 33.37; H, 3.84; N, 10.50.

N-(2-Chloroethyl)-N-(4-chlorobutyl)-glycine Ethyl Ester (No. 744) (XXXXIII)—It was obtained from (XXXXV) by the same procedure as for (XXXXII). Picrylsulfonate, m.p. 128~129° (from EtOH). *Anal.* Calcd. for $C_{16}H_{22}O_{11}N_4Cl_2S$: C, 34.98; H, 4.04; N, 10.10. Found: C, 35.01; H, 4.12; N, 10.29.

N-(2-Chloroethyl)-N-(3-chloropropyl)-glycine (No. 758) (XXXXVI)—The crude hydrochloride of XXXXII (5 g.) was dissolved in 34% HCl (50 cc.) and heated at 80° for 3 hr. Water was distilled off and the residue was converted into the picrylsulfonate, m.p. 165~166° (from EtOH). *Anal.* Calcd. for $C_{13}H_{16}O_{11}N_4Cl_2S$: C, 30.76; H, 3.15; N, 11.04. Found: C, 30.90; H, 3.35; N, 10.51.

N-(2-Chloroethyl)-N-(4-chlorobutyl)-glycine (No. 743) (XXXXVII)—It was obtained from the crude hydrochloride of (XXXXIII) by the same procedure as for (XXXXVI). Picrylsulfonate, m.p. 101~102° (from EtOH). *Anal.* Calcd. for $C_{14}H_{18}O_{11}N_4Cl_2S$: C, 32.26; H, 3.48; N, 10.77. Found: C, 31.96; H, 3.68; N, 10.64.

N-(2-Bromoethyl)-N-methyl-3-bromopropylamine (No. 707) (IX)— PBr_3 (60 g.) was added dropwise into a solution of 3-[(2-hydroxyethyl)methylamino]-1-propanol (34 g.) in benzene (150 cc.) under chilling in ice-water and the mixture was heated at 80° for 3 hr. Benzene was removed and the residue was dissolved in water. After being basified with NaOH, the solution was extracted with Et_2O . The extract was dried over anhyd. K_2CO_3 and treated with dry HCl. A hygroscopic hydrochloride, m.p. 96~97° precipitated. Picrate, m.p. 67~70° (from EtOH). *Anal.* Calcd. for $C_{12}H_{16}O_7N_4Br_2$: C, 29.53; H, 3.30; N, 11.48. Found: C, 29.80; H, 3.28; N, 11.50.

N-(2-Chloroethyl)-N-(3-chloropropyl)-isoxazolidinium Picrylsulfonate (No. 697)—Thirty g. of 35% H_2O_2 was gradually added into Ac_2O (30.6 g.) previously cooled in an ice-water bath, into which a solution of the hydrochloride of (XXIII) (27 g.) in water (150 cc.) was added in drops at 0°. The reaction mixture was warmed to 30° and kept at that temperature for 30 min. After addition of conc. HCl (10 cc.), water was removed *in vacuo* below 30°. The residue was converted into the picrylsulfonate, which was crystallized from MeOH to m.p. 115~116°. *Anal.* Calcd. for $C_{17}H_{19}O_{10}N_4Cl_2S$: C, 33.21; H, 3.78; N, 11.06. Found: C, 33.47; H, 3.83; N, 11.16.

N-(2-Chloroethyl)-N-methyl-4-chlorobutylamine N-oxide (No. 733)—Thirty-five g. of 35% H_2O_2 was slowly added into Ac_2O (30 g.) under chilling in a freezing mixture and the reaction mixture was stirred at the same temperature for 4 hr. (Solution A). The hydrochloride of (VI) (45 g.) was dissolved in water, and after addition of K_2CO_3 at -10° it was extracted with Et_2O (300 cc.) (Solution B). The solution A was then dropped into the solution B below 20° and kept standing at 20° with stirring. After addition of conc. HCl (10 cc.), the mixture was distilled *in vacuo* below 20° and the residue was treated with picrylsulfonic acid. Picrylsulfonate, m.p. 120~121° (from EtOH). *Anal.* Calcd. for $C_{13}H_{18}O_{10}N_4Cl_2S$: C, 31.64; H, 3.67; N, 11.36. Found: C, 31.65; H, 3.69; N, 11.23.

N-(2-Chloroethyl)-N-methyl-pyrrolidinium Picrate by Incubation of (VI)—Hydrochloride of (VI) (10 g.), dissolved in water (100 cc.), was added with NaHCO_3 (5 g.) and the mixture was incubated at 37° for 10 min. The solution was added with picric acid. Picrate, m.p. $177\sim 178^\circ$ (from EtOH). It showed no depression on admixture with the synthesized specimen (No. 701). Yield of the purified picrate, 80%. *Anal.* Calcd. for $\text{C}_{13}\text{H}_{18}\text{O}_7\text{N}_4\text{Cl}$: C, 41.33; H, 4.80; N, 14.84; Cl, 9.38. Found: C, 41.60; H, 4.96; N, 15.03; Cl, 9.40.

Measurement of Cl^- -Liberation and Thiosulfate Uptake—Titration was carried out at 37° by the procedure already reported.³⁾

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Summary

Twenty-three derivatives of N-alkyl-N-2-chloroethyl-N- ω -chloroalkylamine were prepared. It was proved that these compounds are completely monofunctional biological alkylating agents from the estimation of reaction velocity *in vitro*, but nevertheless they exhibit a strong antitumor effect on rats bearing Yoshida sarcoma. The fact is very interesting from the point of the mode of action of nitrogen mustards.

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3) I. Aiko: This Bulletin, 9, 350 (1961).

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64. Kozo Okada: Studies on the Utilization of Safrole as a Medicinal Raw Material. XV.*¹ A Synthesis of 3-Methyl-8,9-methylenedioxy-1H,6H-5,10b-propano-2,3,4,4a-tetrahydrophenanthridine.

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In this paper is described a synthesis of the title compound (V) starting from safrole. As morphinan¹⁾ (A) represents the fundamental skeleton of morphine and related alkaloids, crinine²⁾ (B) represents the skeleton of the Amaryllidaceae alkaloids derived from 5,10b-ethanophenanthridine, such as crinine,²⁾ powelline³⁾ and buphanidine.⁴⁾ These alkaloids possess a strong Morphine-like analgesic action.⁵⁾

Even without methylenedioxy group, the skeleton (C) of which derivative, 9-methoxy-1H,6H-5,10b-propano-2,3,4,4a-tetrahydrophenanthridine,^{*3} was first synthesized by Sugimoto and Kugita⁶⁾ has not yet met in the natural alkaloid. However, the expression (C)

*¹ Part XIV. T. Fujisawa: Yakugaku Zasshi, 79, 783 (1959).

*² Nukui, Koganei, Tokyo (岡田光三).

*³ Sugimoto named this compound 3-methyl-9-aza-morphinan.

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5) N.B. Eddy *et al.*: Bull. World Health and Metabolic Diseases, 14, 353 (1956). (C.A., 50, 15944c (1956)).

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