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[54. Ken-ichi Sawatari : Studies on Carcinostatic Substances. XXXVIII.*1 Chemical and Antitumor Properties of Monofunctional Derivatives of Nitrogen Mustard.

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It has been shown that most of the derivatives of nitrogen mustard inhibit tumor growth, provided that the compound contains at least two 2-chloroethyl groups which are of a certain level of chemical reactivity, from which observation Haddow's well-known hypothesis of cross-linking theory was deduced.[1] It is said that bifunctional alkylating agents cross-link molecules of DNA, twisting their helical structure and thereby interfere in the cell metabolism of tumor.[2]

In respect to chemical reactivity, it has also been known from the past experiences in this field of investigation that the 2-chloroethyl group, incapable of liberating approximately one molar equivalent of chloride ion within 1 hour by incubation of its neutral solution at 37°, does not contribute to manifestation of antitumor action of the compound.[3] For example, N-methyl-3,3'-dichlorodipropylamine (No. 698) and N,N'-bis(3-chloropropyl)-N,N'-diphenylethylenediamine[4] are hardly hydrolyzed under this *in vitro* condition and do not exhibit any antitumor activity.

However, in 1950, Roberts and Kon,[4] and Ross[5] reported the antitumor effect of N-2-chloroethyl-N-3-chloropropylaniline on Walker rat carcinoma and it was also observed recently by the present author that N-methyl-N-2-chloroethyl-3-chloropropylamine[6] (No. 557) showed an antitumor action as strong as that of N-methyl-2,2'-dichlorodiethylamine (HN_2) on Yoshida sarcoma and a series of rat ascites hepatomas.

This is very interesting because it is clearly shown in Table II that the liberation of chloride ion or thiosulfate uptake *in vitro* of the compound (No. 557) in a neutral aqueous solution stops at about one molar equivalent in spite of being incubated for as long as 60 minutes. Needless to say, the liberation or uptake of one molar equivalent should be due to one 2-chloroethyl group of this compound and not to 3-chloropropyl group. Determination of liberation rate of chloride ion in its solution mixed with DNA resulted also in approximately one molar equivalent. These results may be easily understood by the fact that 3-chloropropyl group does not transform into any reactive intermediate corresponding to the aziridinium form of 2-chloroethyl group, and from these experimental data, this compound should be really regarded as a monofunctional compound.

However, one thing to be noted is that the initial velocity of alkylation of No. 557 determined by thiosulfate uptake *in vitro* seems to be faster than the ordinary monofunctional derivatives as shown in Table II, in which case their aziridinium intermediates are more stable in solution due to their stronger basicity.

Therefore, a question arose as to whether or not a compound having only one

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1) E. Boyland, *et al.* : Brit. J. Cancer, **2**, 17 (1948); J. H. Burchenal, *et al.* : Cancer, **1**, 399 (1948).
2) R. T. Goldacre, *et al.* : Nature, **163** (1949); P. Alexander : *Ibid.*, **169**, 226, 572 (1952).
3) M. Bergmann, *et al.* : J. Org. Chem., **11**, 518 (1946); C. J. Swain : J. Am. Chem. Soc., **69**, 2971 (1947); B. Cohen, *et al.* : *Ibid.*, **70**, 281 (1948).
4) R. Kon, J. Roberts : J. Chem. Soc., **1950**, 978.
5) W. C. J. Ross : *Ibid.*, **1950**, 2257.
6) E. R. H. Jones, W. W. Wilson : *Ibid.*, **1949**, 549.

2-chloroethyl group would be effective as antitumor agent if its initial velocity of alkylation were as fast as that of the bifunctional derivative.

To answer this question, some new monofunctional derivatives of reduced basicity which might have alkylating reactivity as high as the bifunctional ones were prepared. The process of synthesis and their chemical and antitumor properties are shown in Chart 1 and in Tables I and II.

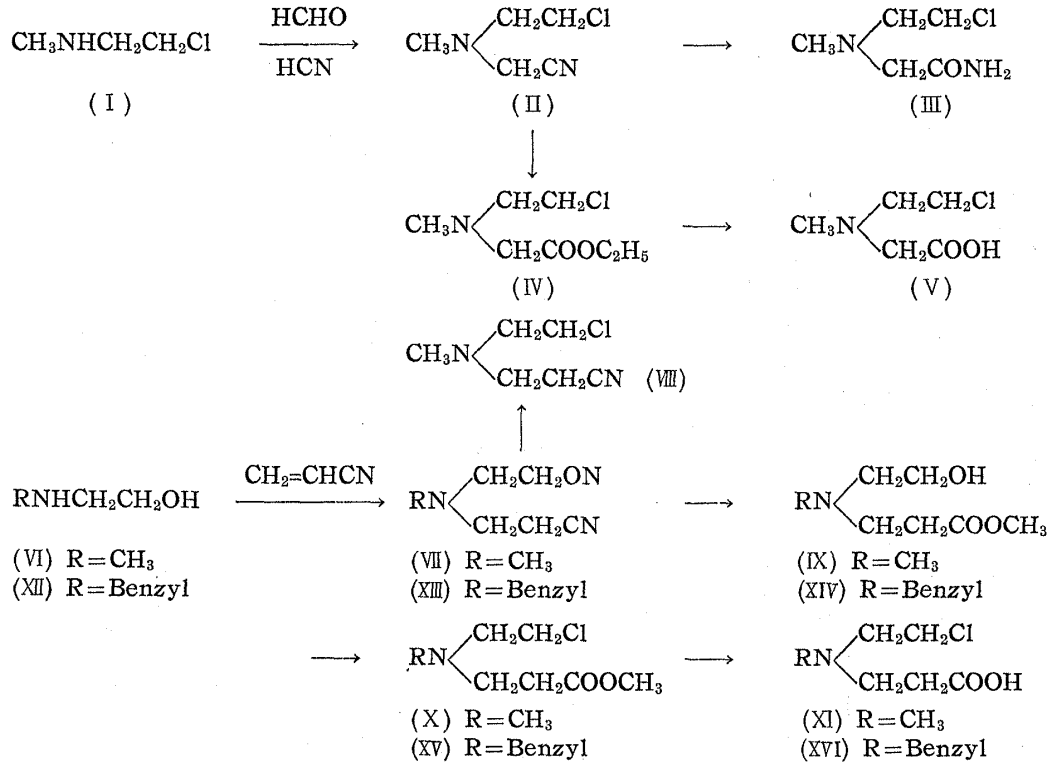

Chart 1.

Table I. Biological Properties of Compounds

Compd. No.	Compound	Salt	LD_{50}[a]	MTD[b]	MED[c]	MEC[d]
557	$CH_3N(CH_2CH_2Cl)(CH_2CH_2CH_2Cl)$	Picrate	50	10	0.5	1×10^{-5}
698	$CH_3N(CH_2CH_2CH_2Cl)(CH_2CH_2CH_2Cl)$	〃	175	100	100	—
673	$CH_3N(CH_2CH_2Cl)(CH_2CH_2COOCH_3)$	Hydrochloride	75	5	—	—
676	$CH_3N(CH_2CH_2Cl)(CH_2CH_2COOH)$	〃	75	50	—	—
679	$C_6H_5CH_2N(CH_2CH_2Cl)(CH_2CH_2COOCH_3)$	〃	75	50	—	—
684	$C_6H_5CH_2N(CH_2CH_2Cl)(CH_2CH_2COOH)$	〃	75	50	—	—
685	$CH_3N(CH_2CH_2Cl)(CH_2COOH)$	Picrylsulfonate	75	50	—	—
686	$CH_3N(CH_2CH_2Cl)(CH_2COOC_2H_5)$	〃	175	100	—	—

a) mg./kg. i.p. on rat.
b) Maximum tolerance dose on rat. mg./kg. i.p.
c) Minimum effective dose on Yoshida sarcoma. mg./kg. i.p. [M. Ishidate, *et al.*: Gann, **44**, 342 (1953)]
d) Minimum effective concentration on *in vitro*-cultured Yoshida sarcoma cell. m*M*. [M. Ishidate, *et al.*: This Bulletin, **7**, 873 (1959)]

Table II. Cl^- Liberation and Thiosulfate Uptake in $NaHCO_3$-buffered Solution at 37°

No.	Salt	Cl^- (molar equiv.) (min.)				S_2O_3 Uptake (molar equiv.) (min.)			
		10	20	30	60	10	20	30	60
557	picrate[a]	0.91	0.91	0.91	0.93	0.86	0.93	0.93	0.93
90[f]	hydrochloride[a]	0.97	0.97	0.97	0.97	0.56	0.78	0.87	0.94
684	hydrochloride[b]	0.93	0.93	0.93	0.97	1.00	1.00	1.00	1.00
679	hydrochloride[c]	0.91	0.93	0.95	0.95	0.88	0.92	0.88	0.88
685	picrylsulfonate[d]	0.91	0.95	0.97	0.97	0.84	0.89	0.92	0.92
686	picrylsulfonate[e]	0.62	0.79	0.92	1.00	0.43	0.72	0.85	0.87
676	hydrochloride[d]	0.68	0.79	0.87	0.91	0.67	0.88	0.93	0.95

a) in H_2O *b*) in 20% MeOH *c*) in 50% MeOH *d*) in 20% Me_2CO
e) in 10% Me_2CO *f*) 2-Chlorotriethylamine.

As expected, the initial velocity of aziridinium formation and alkylation determined by thiosulfate uptake of some of these compounds match those of the bifunctional derivatives and yet they were proved to have no antitumor activity on Yoshida sarcoma.

It can probably be said that the presence of a bifunctional alkylating activity is still indispensable for this kind of compounds in order for them to manifest their antitumor activity, although the reactivity of the one of the two may be too small to be estimated by the *in vitro* reactions if the other had sufficient activity for alkylation.

Experimental

N-2-Chloroethyl-N-methyl-3-chloropropylamine (No. 557)——The preparation was carried out after the method of Jones.[6]
Picrate: m.p. 75°. *Anal.* Calcd. for $C_{12}H_{16}O_7N_4Cl_2$: C, 36.10; H, 4.04; N, 14.04. Found: C, 36.09; H, 3.93; N, 13.88.

N-Methyl-3,3′-dihydroxydipropylamine——A mixture of 3,3′-dihydroxydipropylamine (72 g.), 32% HCHO (65 g.), and 85% HCOOH (39 g.) was heated at 80° for 2 hr. and the product was fractionated. $b.p_{12}$ 145~150°. Yield, 73 g.

N-Methyl-3,3′-dichlorodipropylamine (No. 698)——The above hydroxyamine (73 g.) was chlorinated with $SOCl_2$ (160 g.) in benzene (200 cc.) at 80°. Benzene was distilled off and the residue was dissolved in H_2O. After basification and extraction with Et_2O, it was fractionated *in vacuo*. $b.p_{23}$ 104~106°.
Picrate: m.p. 71°. *Anal.* Calcd. for $C_{13}H_{18}O_7N_4Cl_2$: C, 37.78; H, 4.39; N, 13.58. Found: C, 38.66; H, 4.42; N, 13.51.

2-(N-Methyl-2-chloroethylamino)acetonitrile (II)—N-Methyl-2-chloroethylamine hydrochloride (I) (15 g.) dissolved in H_2O (50 cc.) was added into a mixture of NaCN (4.9 g.) and H_2O (30 cc.), at below 0°. To this mixture 37% HCHO (8.2 g.) was added at the same temperature. After standing for 30 min. at 0°, the reaction mixture was extracted with Et_2O (200 cc.) and the Et_2O extract was used as such for the following reaction because of extreme lability of (II).

2-(N-Methyl-2-chloroethylamino)acetamide (III)——Into the above Et_2O solution of (II), preliminarily cooled in ice water, 60% H_2SO_4 (60 cc.) was added gradually. After standing overnight at room temperature, the mixture was poured on crushed ice, neutralized with NaOH, and extracted with benzene. Dry HCl was passed through the extract and the hydrochloride of (III) separated as a pasty precititate.
Picrylsulfonate: m.p. 86°. *Anal.* Calcd. for $C_{11}H_{14}O_{10}N_5ClS$: C, 29.77; H, 3.18; N, 15.78. Found: C, 29.22; H, 2.97; N, 15.73.

N-Methyl-N-(2-chloroethyl)glycine Ethyl Ester (IV) (No. 686)——The above Et_2O extract of (II) was added with EtOH (100 cc.) and then saturated with dry HCl at 0°. The mixture was gradually warmed to 70° to remove Et_2O and kept at 70° for 2 hr. more. After removal of NH_4Cl by filtration, EtOH was distilled off and the residue was converted to its picrylsulfonate of m.p. 166~167°. *Anal.* Calcd. for $C_{13}H_{17}O_{11}N_4ClS$: C, 33.02; H, 3.62; N, 11.87. Found: C, 33.16; H, 3.55; N, 11.55.

N-Methyl-N-(2-chloroethyl)glycine (V) (No. 685)——The crude hydrochloride of (IV) (10 g.), obtained as the residue of distillation of EtOH, was dissolved in 10*N* HCl (100 cc.) and warmed at 80° for 3 hr. H_2O was distilled off *in vacuo* and the residue was isolated as a crystalline picrylsulfonate, which was recrystallized from 30% MeOH. m.p. 97°. *Anal.* Calcd. for $C_{11}H_{13}O_{11}N_4ClS$: C, 29.70; H, 2.95; N, 12.19. Found: C, 29.73; H, 3.16; N, 12.40.

3-(N-Methyl-2-hydroxyethylamino)propionitrile (VII)——A mixture of acrylonitrile (75 g.) and 2-methylaminoethanol (VI) (70 g.) was warmed at 60~80° with stirring for 12 hr. and the product was fractionated *in vacuo*. (VII) was obtained as a viscous liquid, $b.p_3$ 116~120°.

Picrate: m.p. 95~97°(from MeOH). *Anal.* Calcd. for $C_{12}H_{15}O_7N_5$: C, 40.31; H, 4.23; N, 19.16. Found: C, 40.33; H, 4.34; N, 9.71.

3-(N-Benzyl-2-hydroxyethylamino)propionitrile (XIII)——This was obtained from 2-benzylaminoethanol (XII) and acrylonitrile by the same procedure as described above. $b.p_3$ 170~180°. Picrate: m.p. 105°. *Anal.* Calcd. for $C_{18}H_{18}O_8N_5$: C, 49.97; H, 4.20; N, 16.21. Found: C, 50.29; H, 4.38; N, 15.99.

3-(N-Methyl-2-chloroethylamino)propionitrile (VIII)——Into a mixture of (VII) (10 g.) and benzene (50 cc.), $SOCl_2$ (20 g.) was added with ice cooling and the reaction mixture was warmed gradually up to 40°, at which temperature a violent reaction occurred. After the violent reaction ceased, the mixture was kept at 70° for 3 hr. and the solvent and $SOCl_2$ were removed by distillation. The residue was converted into its picrate of m.p. 102~105°. *Anal.* Calcd. for $C_{12}H_{14}O_6NCl$: C, 38.33; H, 3.75; N, 18.64. Found: C, 37.56; H, 3.75; N, 17.79.

N-Methyl-N-(2-hydroxyethyl)-β-alanine Methyl Ester (IX)——a) A solution of (VII) (10 g.) in MeOH (100 cc.) was saturated with dry HCl and refluxed for 5 hr. After removal of the separated NH_4Cl by filtration, the solvent was distilled off, the residue was again dissolved in H_2O, neutralized with NaOH, and extracted with benzene. After evaporation of benzene, the residue was fractionated *in vacuo*. $b.p_3$ 93~95°(50% yield).

b) A mixture of (VI) (75 g.) and methyl acrylate (86 g.) was heated on a steam bath for 5 hr. and the product was fractionated. Yield was more than 90%.

N-Benzyl-N-(2-hydroxyethyl)-β-alanine Methyl Ester (XIV)——Obtained by the method similar to that for (IX). It distilled at 150~165°/3 mm.Hg, but partial decomposition occurred during distillation. For the following experiment, the crude product was used without purification.

N-Methyl-N-(2-chloroethyl)-β-alanine Methyl Ester (X) (No. 673)——Into a solution of (IX) (10 g.) in benzene (100 cc.), dry HCl was passed. To this mixture, $SOCl_2$ (20 g.) was added dropwise and the whole reaction solution was heated at 80° for 2 hr. After evaporation of benzene and $SOCl_2$, the residue was kept in an ice box until it crystallized (about 5 days), m.p. 105~107°(from AcOEt). *Anal.* Calcd. for $C_7H_{13}O_2NCl_2$: C, 38.90; H, 6.98; N, 6.48. Found: C, 38.67; H, 6.92; N, 6.22.

N-Benzyl-N-(2-chloroethyl)-β-alanine Methyl Ester (XV) (No. 679)——The hydrochloride of (XV) was obtained by the same procedure as in case of (X) but the chlorination was carried out by heating at 70° for 4 hr., m.p. 106~107°(from AcOEt). *Anal.* Calcd. for $C_{13}H_{19}O_2NCl_2$: C, 53.44; H, 6.55; N, 4.79. Found: C, 53.29; H, 6.66; N, 4.87.

N-Methyl-N-(2-chloroethyl)-β-alanine (XI) (No. 676)——A solution of (X) (10 g.) in 10*N* HCl (100 cc.) was heated at 75° for 3 hr. The solution was shaken with activated carbon and filtered. The filtrate was evaporated *in vacuo* to dryness and the residue was kept in ice box until it became crystalline (about 3 days), m.p. 101~102°(from AcOEt). *Anal.* Calcd. for $C_6H_{13}O_2NCl$: C, 35.64; H, 6.48; N, 6.93. Found: C, 35.50; H, 6.59; N, 7.04.

N-Benzyl-N-(2-chloroethyl)-β-alanine (XVI) (No. 684)——Obtained by the same procedure as in case of (XI), m.p. 160°(from AcOEt). *Anal.* Calcd. for $C_{12}H_{17}O_2NCl_2$: C, 51.70; H, 6.75; N, 5.03; Cl, 25.48. Found: C, 51.87; H, 6.23; N, 5.02; Cl, 25.20.

Determinaton of Chloride Liberation and Thiosulfate Uptake——Titration was carried out by the method already reported by Ishidate, *et al.*[7]

Determination of Chloride Liberation of No. 557 in a Solution of DNA——A mixed solution of No. 557 and DNA (from herring sperm) was prepared, in which the concentrations of the two components were respectively 0.01*M* and 0.15%. The whole solution was buffered with $NaHCO_3$ and incubated at 37° for 1 hr. The liberated Cl^- was potentiometrically titrated with 0.01*N* $AgNO_3$. Rate of liberation was 0.92 molar equivalent in case of No. 557 and 1.66 molar equivalent in case of N-methyl-2,2'-dichlorodiethylamine (HN_2).

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Summary

Supported by the fact that N-methyl-N-2-chloroethyl-3-chloropropylamine exhibited a strong antitumor effect on Yoshida sarcoma or series of rat ascites hepatomas, eight derivatives of nitrogen mustard were newly synthesized, which have only one but highly reactive 2-chloroethyl group in a molecule. Antitumor test of these compounds on Yoshida sarcoma was, however, negative.

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