Anal. Calcd. for $C_{38}H_{40}O_5N_2$: C, 75.49; H, 6.62. Found : C, 75.06; H, 6.84.

Summary

Upon the assumption that if any base of the trilobine type (VI) is derived from oxyacanthine (III, R=H), this base must reasonably be identical with either trilobine or isotrilobine, O-methylanhydrodemethyloxyacanthine (VI) was prepared. It was found, however, that the base (VI) so obtained agreed with neither trilobine nor isotrilobine itself, and was an optical isomer of these. On comparison of the optically inactive methyl methine base (VII), obtained by the elimination of the two asymmetric centers in the molecule of (VI), with the optically inactive methyl methine base derived from either trilobine or isotrilobine, both were confirmed to be identical. Accordingly, it is suggested that trilobine and isotrilobine must be optically isomeric with each other, both having the same structure (VI).

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50. **Kozo Maruyama** : Effect of Nitrogen Mustard N-Oxides and the Related Compounds upon Viscosity of a Sodium Polymetacrylate Solution*.

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In 1952, the paper had been published by Alexander[1] which described that viscosity of the neutral solution of polyacrylic acid, like desoxyribonucleic acid, fell markedly when it was kept with the bi- or poly-functional alkylating agents such as nitrogen mustard (HN_2) or tris(ethylenimino)-S-triazine (TEM) at 19° for a certain length of time.

It was also suggested that such decrease in viscosity was caused by the simultaneous alkylation of the neighbouring two acid residues of a desoxyribonucleic acid molecule or a polyacrylic acid molecule by the twin β-chloroethyl groups of the compound under formation of ring esters. On the contrary, in case of the monofunctional reagent, the esterification may occur at random on acid residues and so they had less influence on viscosity of the solution. This fact was supposed to have a certain relation with the biological activity of the compounds.

Nitrogen mustard N-oxides, which had been found by us to have remarkable anticancer effectiveness, were already shown to react with anions as monofunctional alkylating agents *in vitro*, forming N,N-alkyl-β-chloroethyl-O-(A)-ethylhydroxylamines as end products (A : anion)[2].

The aim of this experiment was to decide whether the nitrogen mustard N-oxides belong to the monofunctional alkylating agent or not from the view point of viscosity depression.

As a reaction mixture, a dilute solution containing polymetacrylic acid equivalent to 0.1 *mM* calculated as a monomer and 0.05 *mM* of the alkylating agent was tested in this experiment. After the solution was incubated at 18° for 24 hrs., its relative visco-

* M. Ishidate, Y. Sakurai : Studies on Cancerocidal Substances. IX.

** 1-26, Nishigahara, Kita-ku, Tokyo (丸山幸三).

1) P. Alexander : Nature, **169**, 226, 572 (1952).

2) I. Aiko, S. Owari, M. Torigoe : J. Pharm. Soc. Japan, **72**, 1297 (1952); S. Owari : This Bulletin, **1**, 353 (1953).

sity at various pressure was determined using Tsuda's viscosimeter[3] at 27°. The depression of viscosity was expressed as a ratio (R), calculated by the following formula :

$$R = \frac{\eta_c - \eta}{\eta_c} \times 100$$

η_c : relative viscosity of the control metacrylate solution at 27°.
η : relative viscosity of the test solution at 27°.

It could not be avoided in this experiment that the test solution contained a small amount of NaCl which was produced either by alkylation or by neutralization of the amine hydrochloride. However, the control experiments proved that such an amount of inorganic salt had little influence upon the rate of viscosity depression.

Twenty of the compounds, including the monofunctional, the polyfunctional nitrogen mustards and their N-oxides are discussed in this paper and the results obtained are shown in Table I and Fig. 1.

Table I.

Compound	Rate of depression of viscosity (R) at each pressure* at 27°C			Esterified –COOH in mol. equiv.	Liberated Cl^- in mol. equiv. to an amine**
	7.0 cm.	11.0 cm.	14.5 cm.		
$(CH_3)_2{=}N{-}C_2H_4Cl$	11	10	9	0.2	1.0
$(C_2H_5)_2{=}N{-}C_2H_4Cl$	7	7	7	0.2	1.0
$CH_3{-}N{<}^{C_2H_4Cl}_{C_2H_4OH}$	5	5	5	0.2	1.0
$(CH_3)_2{=}N{-}C_2H_4Cl \rightarrow O$	3	3	3	0.2	1.0
$(C_2H_5)_2{=}N{-}C_2H_4Cl \rightarrow O$	6	6	6	0.2	1.0
$CH_3{-}N{=}(C_2H_4Cl)_2 \rightarrow O$	6	6	6	0.2	1.0
$ClC_2H_4{-}N\langle\rangle N{-}C_2H_4Cl$	13	13	13	0.1	1.0
$n\text{-}C_5H_{11}{-}N{=}(C_2H_4Cl)_2$	14	14	13	0.1	1.0
$CH_3{-}N{=}(C_2H_4Cl)_2$	23	23	22	0.5	1.8
$C_2H_5{-}N{=}(C_2H_4Cl)_2$	23	23	23	0.3	1.8
$iso\text{-}C_3H_7{-}N{=}(C_2H_4Cl)_2$	25	25	24	0.3	1.8
$CH_2{=}CHCH_2{-}N{=}(C_2H_4Cl)_2$	24	24	23	0.3	1.8
$iso\text{-}C_5H_{11}{-}N{=}(C_2H_4Cl)_2$	25	25	24	0.3	1.6
$\langle H \rangle{-}N{=}(C_2H_4Cl)_2$	26	26	25	0.3	1.4
$N{\equiv}(C_2H_4Cl)_3$	23	23	22	0.4	2.2
$(ClC_2H_4)_2{=}N{-}C_2H_4{-}N{=}(C_2H_4Cl)_2$	24	24	23	0.4	1.4
$iso\text{-}C_5H_{11}{-}N{=}(C_2H_4Cl)_2 \rightarrow O$	27	27	26	0.3	1.0
$\phi\text{-}CH_2{-}N{=}(C_2H_4Cl)_2 \rightarrow O$	31	31	31	0.5	1.0
$N{\equiv}(C_2H_4Cl)_3 \rightarrow O$	29	29	28	0.5	1.0
$(ClC_2H_4)_2{=}N{-}C_2H_4{-}N{=}(C_2H_4Cl)_2$ (↓O, ↓O)	28	28	27	0.5	2.5

Relative viscosity of 0.054% polymetacrylate solution used herein η_c : 1.36 at 11.0 cm. H_2O press.

* Height of columm of solution in cm.

** Cl ion, initially existing in the solution as an amine hydrochloride, was deducted from the titration value.

3) S. Tsuda : Kolloid-Z., **45**, 325(1928).

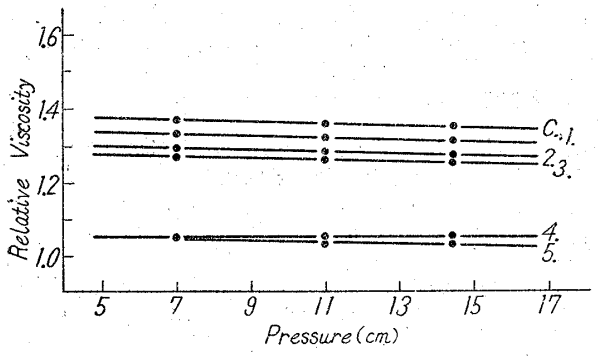


Fig. 1.

1. : $(CH_3)_2{=}N{-}C_2H_4Cl$ ↓ O

2. : $CH_3{-}N{=}(C_2H_4Cl)_2$ ↓ O

3. : $(C_2H_5)_2{=}N{-}C_2H_4Cl$

4. : $CH_3{-}N{=}(C_2H_4Cl)_2$

5. : $C_2H_4{-}N{=}(C_2H_4Cl)_2$

C. : Control

Concerning the effect on the decrease in viscosity, these results coincided well with those of Alexander and the N-oxides, regardless of their functionality, belonged in general to the monofunctional in this respect, with a few exceptions. However, in these experiments, the free carboxyl groups remained unesterified and chlorine ion liberated were either determined by titration together with measuring of viscosity and the conclusion is considered to be that the bi- or poly-functional compounds exhibited dominant influences upon viscosity not only by ring-forming or cross-linking abilities as having been stated by many authors but also by their surpassing velocity of esterifying reaction compared with the monofunctional or the N-oxides as shown in Table I.

Consequently it was tried in succeeding experiments to exclude the latter factor of viscosity depression in order to make the matter simple and clear. It was found that, adopting the necessary concentration of the reactants and the proper time length of incubation in each case, the solution of sodium polymetacrylate could be esterified to the equal degree, i.e. to 30% of all carboxyl groups, in all cases. Each of the reaction mixtures was then diluted to a similar concentration after completion of the reaction and determined as to its relative viscosity in the same manner.

As shown in Table II, the bi- or poly-functional compounds were definitely distinguished from the monofunctional in respect to viscosity depression even when the rate of esterification was just equal in both cases, and the N-oxides belonged all the same to the latter. If the test solution containing methyl-bis(β-chloroethyl)amine N-oxide was incubated for one more day after the viscosity determination, an amorphous precipitate began to separate in the solution, the analytical data of which coincided well with those of an ester having the following constitution :

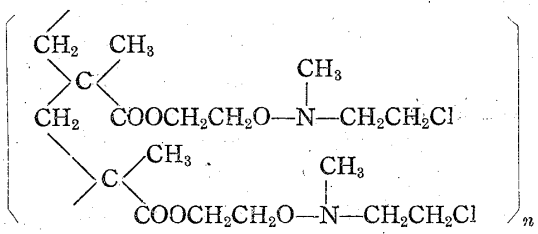


Table II.

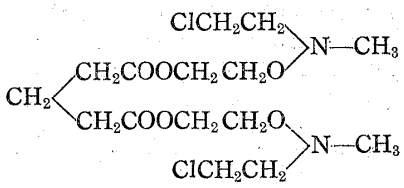
Compound	Esterified –COOH in mol. equiv.	Conditions of esterification: Concentration in *mM.*	Time in hr.	R in each pressure at 27°C: 5.0 cm.	10.0 cm.	17.0 cm.
$(CH_3)_2{=}N{-}C_2H_4Cl$	0.3	500	24	27	27	27
$(C_2H_5)_2{=}N{-}C_2H_4Cl$	0.3	500	24	26	26	26
$CH_3{-}N{=}(C_2H_4Cl)_2$ ↓ O	0.3	500	7	23	23	23
$CH_3{-}N{=}(C_2H_4Cl)_2$	0.3	5	4	38	38	38
$C_2H_5{-}N{=}(C_2H_4Cl)_2$	0.3	5	4	39	39	39

η_c : 1.76 at 10.0 cm.

However, nitrogen mustard N-oxides showed in reality very promising effects upon the experimental and clinical tumors in spite of the widely recognized opinion that the monofunctional alkylating agents have little or no anticancer effectiveness. Therefore, it is not too much to say that the results obtained herein gave support to our assumption that the N-oxides are gradually reduced *in vivo* to the bifunctional nitrogen mustards and, furthermore, it is due to the selective reduction *in vivo* according to the redox-potential of the milieu that they exhibit the wider range of dose between the minimum therapeutic and the maximum tolerated doses than the corresponding nitrogen mustards.

In Table II, isoamyl-bis(β-chloroethyl)amine N-oxide and benzyl-bis(β-chloroethyl)-amine N-oxide showed the exceptional depression of viscosity which lay almost between the mono- and bi-functional compounds. It was of course difficult to account for the reason but the two compounds had a stronger tendency to be reduced than the other N-oxides and it may be said that the reduction took place partially in these cases even *in vitro* during incubation. In fact, the half-wave reduction potential of the two compounds, measured by polarograph, was lower than those of the other N-oxides[5].

It was also confirmed that sodium glutarate, like the polymetacrylate, could be esterified by methyl-bis(β-chloroethyl)amine N-oxide, yielding an ester which was identified by analysis to have the following constitution:



When sodium glutarate was esterified with methyl-bis(β-chloroethyl)amine (HN_2) instead of its N-oxide, bis[β-(methyl-β-oxyethylamino)ethyl]glutarate was obtained as a reaction product.

Although this reaction was far more complicated than in the case of its N-oxide so that the reaction products excepting an ester above-mentioned could not be identified, it can probably be said that the cross-linking plays the more leading role in the mode of alkylation of the bifunctional nitrogen mustards than the ring-ester formation as stated by Alexander.

Experimental

1) Preparation of the Test Solution—Polymetacrylic acid was prepared after Brown *et al.*[6] by heat condensation of the monomer at 130° for 6 hrs. and the polymerized acid was washed with ether. It was completely soluble in dil. NaOH. Each test solution for viscosity determination was prepared as follows: 10 cc. of 0.108% solution of sodium polymetacrylate (equivalent to 0.1 *mM* calculated as a monomer) was added with each 0.05 *mM* of the sample of nitrogen mustards hydrochloride, previously dissolved in a small quantity of water and neutralized with 0.05 *mM* NaOH. The mixture was then diluted exactly to 20 cc. and incubated at 18°. After 24 hrs. it was adjusted strictly to pH 7.0 with a test paper and relative viscosity was measured at 27° by Tsuda's apparatus[3].

As the control, 0.054% solution of sodium polymetacrylate was measured as to its relative viscosity at 27°, which was 1.36 in one case (Table I) and 1.76 in the other (Table II), as the polymerized acids used in both experiments were not the same.

2) Influence of NaCl on Viscosity of the Solution—Twenty cc. of the test solution contained at least 0.05 *mM* NaCl which was obtained by neutralization of the amine hydrochloride and, after esterifying reaction, it contained more NaCl. However, it cannot be more than 0.1 *mM* in the total in case of the bifunctional compounds, calculated from the rates of esterification shown in Tables I and II. It is shown in Table III that the value of R was not much influenced by such an amount of the salt.

5) I. Aiko: This Bulletin, **1**, 335 (1953).
6) A. C. Brown, J. Walker: Ann., **274**, 56 (1894).

Table III.

mM of NaCl in 20 cc. of the solution	R in each pressure at 27°C		
	7.0 cm.	11.0 cm.	14.5 cm
0.05	3	3	3
0.10	6	6	6
0.15	8	8	8
0.20	9	9	9

η_c : 1.36 at 11.0 cm.

3) Titration of Free Carboxyl Group Unesterified—The pH of the test solution fell to 6 to 4 at the end of the reaction. It was neutralized by dil. NaOH strictly to pH 9 and then titrated by *N*/100-HCl to pH 3 using a glass-electrode pH-meter. It was confirmed that the pH of a 0.054% solution of sodium polymetacrylate was 9.0 and its titration value of HCl from pH 9 to 3 was nearly equivalent to the amount of the polymetacrylic acid.

4) Titration of Cl^- liberated—The titration was carried out with *N*/100-$AgNO_3$ after Mohr's method for the purpose of prompt operation of the series of the test solutins.

5) An Ester of Polymetacrylic Acid—An amorphous precipitate was obtained as stated above and it was washed repeatedly with water. It showed a strong Beilstein's halogen reaction but had no oxidative property against KI solution. *Anal.* Calcd. for $(C_{18}H_{32}O_6N_2Cl_2)_n$: C, 48.75 ; H, 7.22 ; N, 6.32. Found: C, 48.99; H, 7.17; N, 6.27.

6) Esters of Glutaric Acid—An aqueous solution of glutaric acid (0.01 *M*), NaOH (0.03 *M*), and methyl-bis(β-chloroethyl)amine N-oxide hydrochloride (0.01 *M*) was incubated at 18° for 24 hrs. It soon turned turbid and oil drops separated out at the end of the reaction. The reaction mixture was adjusted to pH 7, and the oil was extracted by ether. It was again dissolved in dil. HCl and a solution of ammonium reineckate added. A dark red reineckate was precipitated and recrystallized from butanol. It melted at 134°(decomp.) and showed a strong Beilstein's halogen reaction but had no oxidative property against a solution of KI. *Anal.* Calcd. for $C_{23}H_{42}O_6N_{14}Cl_2Cr_2S_8 \cdot 3H_2O$: C, 25.18; H, 4.38; Cr, 10.34; H_2O (crystal), 5.37. Found : C, 25.10; H, 4.23; Cr, 10.90; H_2O (crystal), 5.29.

When a similar reaction was carried out, using methyl-bis(β-chloroethyl)amine hydrochloride instead of the N-oxide, a small amount of an ester could be isolated alone as a picrylsulfonate melting at 60°. The most part of the reaction product neither crystallized nor was identified except the unreacted nitrogen mustard and its dimerization product, N,N-bis(methyl-β-chloroethyl)piperazinium dichloride. The picrylsulfonate (m.p. 60°), obtained herein, showed no more halogen reaction. *Anal.* Calcd. for $C_{27}H_{36}O_{24}N_8S_2$: C, 35.22; H, 3.91; N, 12.17. Found : C, 35.22; H, 4.00, N, 12.23.

Summary

Effect of nitrogen mustards and their N-oxides upon viscosity of the dilute sodium polymetacrylate solution was discussed and it was confirmed that only the di- or poly-functional compounds caused a decrease in relative viscosity of the solution while the monofunctional had little influence upon it. The N-oxides, in general, belonged to the latter group of compounds in respect to this effect. The fact may give support to the presumption that the N-oxides are reduced *in vivo* according to the redox potential of the milieu, forming the bifunctional amines and exhibiting the effectiveness on tumors, because the monofunctional has so far been believed to be biologically inactive.

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