Attempts at Synthesizing Compounds Isosteric with Methyl-bis-(β-chloroethyl)amine ("Nitrogen Mustard")

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Methyl-bis-(β-chloroethyl)amine was first synthesized by Prelog and was later used for the synthesis of the analgesic substance dolantin (demerol, pethidin). That this compound is a vesicant with an effect on skin and mucous membranes similar to that exerted by mustard gas was in 1941 observed by Jensen and Lundquist. This property was explained by the fact that methyl-bis-(β-chloroethyl)amine is isoelectronic (or isosteric in a broader sense) with mustard gas, bis-(β-chloroethyl)sulfide:

\[
\begin{align*}
\text{S} & \text{CH}_2 \cdot \text{CH}_2 \text{Cl} \\
& \text{CH}_2 \cdot \text{CH}_2 \text{Cl} \\
\text{bis-(β-chloroethyl)sulfide} & \text{CH}_3 \cdot \text{N} \text{CH}_2 \cdot \text{CH}_2 \text{Cl} \\
& \text{CH}_2 \cdot \text{CH}_2 \text{Cl} \\
\text{(mustard gas)} & \text{methyl-bis-(β-chloroethyl)amine} \\
& \text{("nitrogen mustard")}
\end{align*}
\]

Considerable work was done during World War II on the toxicology and pharmacology of nitrogen mustard with a view to a possible use of this compound as a chemical warfare agent. During this work it was observed that the biological effects both of sulfur mustard and nitrogen mustard show a very striking similarity to those of ionizing radiations. The toxic effect of the mustards is especially pronounced toward bone marrow and lymphoid tissue, and because of this effect intravenous injection of the hydrochloride of "nitrogen mustard" has been used for the treatment of leukaemia and other neoplastic diseases of the hematopoietic system (the very extensive literature on the clinical use has been reviewed by Gellhorn and Jones and Reinhard et al.). From a theoretical point of view, however, the most interesting effect of "nitrogen mustard" is that it is mutagenic. Mutations have been caused in
Drosophila (Auerbach and Robson ⁶ as well as in Neurospore (Horrowitz et al., Tatum ⁸). Several other compounds have been claimed to be mutagenic, but a critical analysis of the results show that a true reversible mutagenic action, similar to that exerted by ultraviolet rays, had until quite recently only been demonstrated for sulfur mustard, nitrogen mustard and a few closely related compounds (cf. Auerbach ⁹ and Jensen, Kølmark and Westergaard ¹⁰).

In connection with a more extensive investigation on the mutagenic effect of chemical substances we wished to try the effect of some compounds isosteric with "nitrogen mustard", viz. the following compounds:

\[
\begin{align*}
\text{III. } & \quad N,N\text{-bis-} \\
& \quad (\beta\text{-chloroethyl})\text{hydrazine} \\
\text{HO} & \quad N \\
\text{CH}_2 & \quad \cdot \text{CH}_2\text{Cl} \\
\text{CH}_2 & \quad \cdot \text{CH}_2\text{Cl} \\
\text{IV. } & \quad \text{bis-(\beta\text{-chloroethyl})} \\
& \quad \text{hydroxylamine} \\
\text{H}_2\text{N} & \quad \text{CH} \\
\text{CH}_2 & \quad \cdot \text{CH}_2\text{Cl} \\
\text{CH}_2 & \quad \cdot \text{CH}_2\text{Cl} \\
\text{V. } & \quad \text{N-fluoro-bis-} \\
& \quad (\beta\text{-chloroethyl})\text{amine} \\
\text{F} & \quad \text{N} \\
\text{CH}_2 & \quad \cdot \text{CH}_2\text{Cl} \\
\text{CH}_2 & \quad \cdot \text{CH}_2\text{Cl} \\
\text{VI. } & \quad 1,5\text{-dichloro-3-amino-} \\
& \quad \text{pentan} \\
\text{H}_2\text{N} & \quad \text{CH} \\
\text{CH}_2 & \quad \cdot \text{CH}_2\text{Cl} \\
\text{CH}_2 & \quad \cdot \text{CH}_2\text{Cl} \\
\end{align*}
\]

Of these the last compound (no. VI) had been synthesized previously by Hahn, Prelog and Cerkovnikov ¹¹ and was prepared according to their directions. It was found to be devoid of mutagenic effect (Jensen, Kirk and Westergaard ¹²). In spite of numerous attempts, we did not succeed in synthesizing the compounds III—V. The hydroxy compounds corresponding to compounds III and IV are known (formed from ethylene oxide and hydrazine and hydroxylamine, respectively ¹³), but treatment of the hydrochlorides of these hydroxyamines with concentrated hydrochloric acid resulted in complete decomposition, ammonium chloride being formed. By treatment with thionyl chloride the hydrochloride of N,N-bis-(\beta\text{-hydroxyethyl})hydrazine gave a dark, viscous, seemingly highpolymeric substance.

From bis-(\beta\text{-chloroethyl})amine the nitrosamine (VII) could be prepared. This could be reduced catalytically in acetic acid solution, but although the hydrazine was presumably formed (the solution reduced cupric hydroxide in the cold), we did not succeed in isolating it in form of a salt or a derivative with a carbonyl compound.
The formation of the hydroxylamine (IV) was also attempted by oxidation of bis-(β-chloroethyl)amine with hydrogen peroxide, but no hydroxylamine was formed in acid or neutral solution, and in alkaline solution the chlorine atoms are split off.

N-Fluoro compounds are only known in the form of perfluoro derivatives. Attempts at preparing simple N-fluoro compounds by reaction of N-chloro- or N-bromo-amines with silver or mercuric fluoride were not successful.

In connection with these experiments we prepared N-chloro-bis-(β-chloroethyl)amine and tetra-β-chloroethyl-methylenediamine (VIII and IX).

VII. N-nitroso-bis-(β-chloroethyl)amine

VIII. N-chloro-bis-(β-chloroethyl)amine

IX. tetra-β-chloroethyl-methylenediamine

EXPERIMENTAL

Microanalyses by Albert Grossmann

_N-Nitroso-bis- (β-chloroethyl) amine_ (VII). To an ice-cold solution of 3.6 g (0.02 mole) of bis-(β-chloroethyl)amine hydrochloride in 5 ml of water was added 1 drop of conc. hydrochloric acid and a solution of 0.7 g of sodium nitrite in 2 ml of water. After standing in an ice-bath for half an hour the nitrosoamine was extracted with ether. The ethereal solution was washed with water and dried with sodium sulfate. After removing the ether _in vacuo_ the nitrosoamine was left as a yellow oil which could not be distilled. Yield 2.5 g = 73 %.

C₄H₅ON₂Cl₂ (171.1) Calc. C 28.08 H 4.71 N 16.38 Cl 41.45
Found 27.84 4.64 16.44 41.49

_N-Chloro-bis- (β-chloroethyl) amine_ (VIII). To an icecold solution of sodium hypochlorite (prepared from 30 g of NaOH in 250 ml of water + 100 g of ice) a solution of 18 g of bis-(β-chloroethyl)amine hydrochloride in 50 ml of water was added. An oil separated. After standing in an ice-bath for a few hours the solution was extracted with ether. The ethereal layer was dried with sodium sulfate, and the ether removed _in vacuo_. The liquid residue was filtered into a Claisen-flask and distilled _in vacuo_. B.p. 110° at 18 mm. Yield 10 g = 55 %.

C₄H₅NCl₃ (176.5) Calc. N 7.94 Cl 60.30
Found 8.10 60.46

On standing the compound is rapidly decomposed with deposition of crystals.

_N,N-Tetra-β-chloroethyl methylenediamine_ (IX). A solution of 3.0 g of bis-(β-chloroethyl)amine hydrochloride in 50 ml of formaldehyde (40 %) was added slowly with stirring to a solution of 1.25 g of KOH in 5 ml of water. The solution was warmed a few minutes
on a steam bath, cooled, and extracted with ether. The ether solution was dried with
$K_2CO_3$ and when the ether was removed in vacuo the amine was left as an oil which could
not be distilled without decomposition. On passing dry hydrogen chloride through the
etheral solution the hydrochloride separated as an oil. The picrate was obtained in crys-
talline form by addition of an aqueous or ethanolic solution of picric acid to the amine
and could be recrystallized from water. M.p. 175°.

\[ C_{21}H_{24}O_{14}N_8Cl_4 \ (754.3) \quad \text{Calc.} \quad N \quad 14.85 \]
\[ \text{Found} \quad 15.01 \]

The melting point of the picrate of bis-(\(\beta\)-chloroethyl)amine is 112—113°.

The maleate was prepared in the following way: To a dry ethanolic solution of the
amine a solution of 2.3 g of maleic acid in 150 ml of dry ether was added. Colourless
crystals separated. After some hours they were filtered and dried. Yield 1.3 g (25 %).
M.p. 129—130°. No pure substance could be isolated from the mother liquor.

\[ C_{17}H_{26}O_2N_2Cl_4 \ (528.2) \quad \text{Calc.} \quad N \quad 5.31 \quad \text{Cl} \quad 28.84 \]
\[ \text{Found} \quad 5.47 \quad 27.77 \]

**SUMMARY**

The following compounds have been synthesized for the first time: N-
nitroso-bis-(\(\beta\)-chloroethyl)amine, N-chloro-bis-(\(\beta\)-chloroethyl)amine and N,N-
tetra-\(\beta\)-chloroethyl-methylenediamine. Attempts were made to synthesize
N,N-bis-(\(\beta\)-chloroethyl)hydrasine, bis-(\(\beta\)-chloroethyl)hydroxylamine and N-
fluoro-bis-(\(\beta\)-chloroethyl)amine, but were unsuccessful.

**REFERENCES**

5. Geilhorn, A., and Jones, L. Am. J. Med. 6 (1949) 188.
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