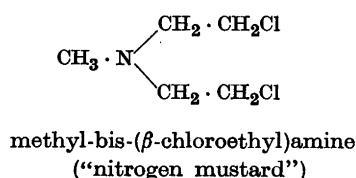
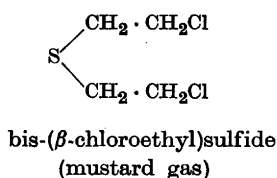


Attempts at Synthesizing Compounds Isosteric with Methyl-bis-(β -chloroethyl)amine ("Nitrogen Mustard")

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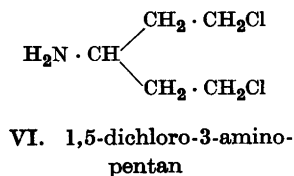
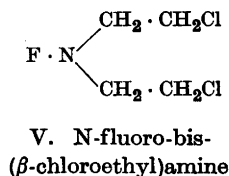
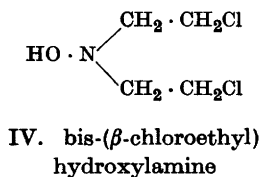
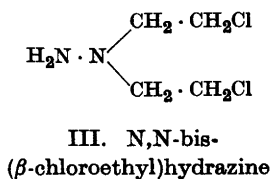
Methyl-bis-(β -chloroethyl)amine was first synthesized by Prelog¹ and was later used for the synthesis of the analgesic substance dolantin² (demerol, pethidin). That this compound is a vesicant with an effect on skin and mucous membranes similar to that exerted by mustard gas was in 1941 observed by Jensen and Lundquist³. 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:



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 radiations⁴. 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.*⁵). 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 Robson⁶ as well as in *Neurospore* (Horowitz *et al.*⁷, Tatum⁸). 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. Auerbach⁹ and Jensen, Kølmark and Westergaard¹⁰).

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:



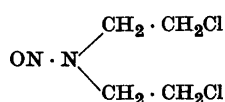
Of these the last compound (no. VI) had been synthesized previously by Hahn, Prelog and Cerkovnikov¹¹ and was prepared according to their directions. It was found to be devoid of mutagenic effect (Jensen, Kirk and Westergaard¹²). In spite of numerous attempts, we did not succeed in synthesizing the compounds III—V. The hydroxy compounds corresponding to compounds III and IV are known (formed from ethylene oxide and hydrazine and hydroxylamine, respectively¹³), 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-(β -hydroxyethyl)hydrazine gave a dark, viscous, seemingly highpolymeric substance.

From bis-(β -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.

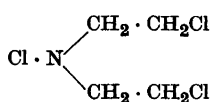
The formation of the hydroxylamine (IV) was also attempted by oxydation of bis-(β -chloroethyl)amine with hydrogen peroxide, but no hydroxylamine was formed in acid or neutral solution, and in alkaline solution the chlorine atoms are split off.

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.

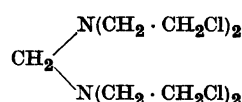
In connection with these experiments we prepared N-chloro-bis-(β -chloroethyl)amine and tetra- β -chloroethyl-methylenediamine (VIII and IX).



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 nitrosamine was extracted with ether. The ethereal solution was washed with water and dried with sodium sulfate. After removing the ether *in vacuo* the nitrosamine was left as a yellow oil which could not be distilled. Yield 2.5 g = 73 %.

$\text{C}_4\text{H}_8\text{ON}_2\text{Cl}_2$ (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 %.

$\text{C}_4\text{H}_8\text{NCl}_3$ (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 3.6 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 ethereal solution the hydrochloride separated as an oil. The picrate was obtained in crystalline 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)	Calc.	N	14.85
	Found	»	15.01

The melting point of the picrate of bis-(β -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.

$C_{17}H_{26}O_8N_2Cl_4$ (528.2)	Calc.	N	5.31	Cl	26.84
	Found	»	5.47	»	27.77

SUMMARY

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

REFERENCES

1. Prelog, V., and Štěpán, V. *Collection Czechoslov. Chem. Commun.* **7** (1935) 93.
2. D.R.P. 679 281 (1939).
3. Jensen, K. A., and Lundquist, F. *Dansk Tids. Farm.* **15** (1941) 201.
4. Gilman, A., and Philips, F. S. *Science* **103** (1946) 409.
5. Gellhorn, A., and Jones, L. *Am. J. Med.* **6** (1949) 188.
Reinhard, E. H., Good, J. T., and Martin, E. J. *Am. Med. Assoc.* **142** (1950) 383.
6. Auerbach, C., and Robson, J. M. *Nature* **157** (1946) 302.
7. Horowitz, N. H., Houlahan, M. B., Hungate, M. G., and Wright, B. *Science* **104** (1946) 233.
8. Tatum, E. L. *Cold Spring Harbor Symposia Quant. Biol.* **11** (1946) 278.
9. Auerbach, C., Robson, J. M., and Carr, J. G. *Science* **105** (1947) 243.
10. Jensen, K. A., Kølmak, G., and Westergaard, M. *Hereditas* **35** (1949) 521.
11. Hahn, V., Cerkovnikov, E., and Prelog, V. *Helv. Chim. Acta* **26** (1943) 1132.
12. Jensen, K. A., Kirk, I., and Westergaard, M. *Nature* **166** (1950) 1019.
13. Knorr, L., and Brownsdon, H. W. *Ber.* **35** (1902) 4474.
Plissow, A. K. *Ukrain. Khim. Zhur.* **3** (1928) 125; *Chem. Zentr.* (1930 : I) 2867.
Jones, L. W., and Burns, G. R. *J. Am. Chem. Soc.* **47** (1925) 2970.

Received July 9, 1951.