# SYNTHESIS OF S-(3-AMINOBUTYL)ISOTHIOUREA AND ITS HETEROCYCLIC DERIVATIVE AND RADIOPROTECTIVE EFFECT OF THESE COMPOUNDS

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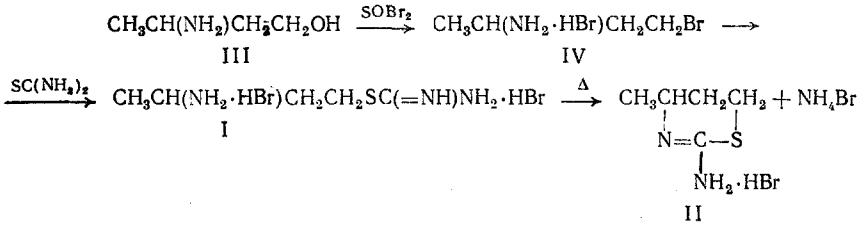
Among the known sulfur-containing organic compounds having radioprotective properties, aminoalkylisothioureas [4-6] are the most effective in reducing radiation injury to mammals.

Study of the metabolism of biologically active aminoalkylisothioureas in mammals has shown that compounds of this class are transformed as soon as within the first hour into heterocyclic compounds of the dihydrothiazinethiazoline series which also have antiradiation activity. The presence of these compounds in mammalian organs and tissues accounts for the radioprotective effect of the corresponding isothioureas [1, 3].

In this study a method is presented for the synthesis of S-(3-aminobutyl)isothiourea dihydrobromide (I) and its cyclization product, 2-amino-4-methyl-5,6-dihydro-4H-1,3-thiazine (II). The starting compound is 3-aminobutanol (III). Reaction of III with $SOBr_2$ in benzene gives 3-amino-1-bromobutane (IV). The dihydrobromide I is obtained with a 72% yield by boiling thiourea and IV in absolute 2-propanol. The cyclization of I and II was accomplished by boiling in water.



## EXPERIMENTAL (CHEMICAL)

The course of the reactions was monitored by thin-layer chromatography on Silufol UV-254 plates in a n-butanol—acetic-acid—water (4:1:1) system. The yield and properties of the synthesized compounds are presented in Table 1.

S-(3-Aminobutyl)isothiourea Dihydrobromide (I). After dissolving 7.6 g (0.1 mole) of thiourea in 200 ml of boiling abs. 2-propanol, 15.3 g (0.1 mole) of IV are added and the mixture boiled for 8 h until the disappearance of thiourea. Acetone is added to the cooled solution. The resulting crystals of I are repeatedly washed with acetone.

2-Amino-4-methyl-5,6-dihydro-4H-1,3-thiazine Hydrobromide (II). A solution of 6.2 g (0.02 mole) of I is boiled in water for 20 h then evaporated to dryness. The residue is treated with hot n-butanol and the solution decanted. A white crystalline residue is formed upon cooling, from which II is obtained by recrystallization from n-butanol..

3-Amino-bromobutane Hydrobromide (IV). A mixture of 34 g (0.2 mole) of 3-amino-1-butanol hydrobromide (III) and 46 g (0.22 mole) of thionyl bromide is kept in anhydrous benzene at 40°C for 24 h. Residual thionyl bromide and benzene are then distilled off in vacuo. The dry residue is recrystallized from anhydrous ethyl acetate, giving IV.

## EXPERIMENTAL (BIOLOGICAL)

The toxicity and radioprotective activity of I and its heterocyclic derivative II were studied in experiments with mammals. The effect of prophylactic administration of these compounds on the hemopoietic system of irradiated animals was also studied.

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TABLE 1. Chatacteristics of Synthesized Compounds

| Compound | mp., °C | Yield, % | Found, % | | | | Empirical formula | Calculated, % | | | | $R_f$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | C | H | N | S | | C | H | N | S | |
| I | 186 | 72 | 19,30 | 4,72 | 13,67 | 10,40 | $C_3H_{15}Br_2N_5S$ | 19,45 | 4,85 | 13,59 | 10,35 | 0,15 |
| II | 153 | 76 | 28,35 | 5,29 | 13,34 | 15,07 | $C_6H_{11}BrN_2S$ | 28,40 | 5,21 | 13,25 | 15,15 | 0,45 |
| IV | 148 | 60 | 31,43 | 7,18 | 9,22 | — | $C_7H_{11}Br_2N$ | 31,40 | 7,21 | 9,17 | — | 0,64 |

TABLE 2. Radioprotective Efficiency of I and II on Prophylactic Administration to Mice Prior to Irradiation of 9.5 G

| Compound | Dose, mg/kg | Time between administration and irradiation | Survival rate, % | Efficiency |
|---|---|---|---|---|
| I | 250 | 0,5 | 42.0±1.62 | 13/31 |
| | | 1 | 50.0±0.84 | 30/60 |
| | | 2 | 27.3±1.02 | 12/44 |
| | | 3 | 16.7±3.26 | 2/12 |
| II | 150 | 1 | 32.2±0.80 | 19/59 |
| | | 2 | 14.3±2.59 | 2/14 |
| | 300 | 0,5 | 18.7±2.50 | 3/16 |
| | | 1 | 26.6±3.05 | 4/15 |
| | | 2 | 20.0±2.96 | 3/15 |
| | | 3 | 14.3±2.59 | 2/14 |
| Control | — | — | 0 | 0/89 |

Note. The numerator is the No. of surviving animals; the denominator is the total No. of animals

The experimental subjects were $F_1$(CBA×C57BL) mice of both sexes weighing 18-22 g.

Determination of the toxicity of the preparations showed that $LD_{16}$, $LD_{50}$, and $LD_{84}$ values were 255, 300, and 355 mg/kg, respectively, for I and 142.5, 162.5, and 187.0 mg/kg for II when the compounds were administered subcutaneously.

In studying the antiradiation activity, the substances investigated were administered to the animal at a dose equal to the $LD_{16}$ in 0.5 ml of physiological solution at various times (0.5, 1, 2, 3 h) before irradiation. The mice were subjected to the effect of $^{60}Co$ γ-rays at a dose of 9.5 G ($LD_{100/30}$) with a dose power of 1.7 G/min. The criterion of damage was the survival of the animals for 30 days.

The effect of the substances studied on the state of the hemopoietic systems was judged according to the No. of cells in the bone marrow of the femur and the weight of the spleen at various time intervals following irradiation (from 4 to 15 days).

It was established that I is less toxic than II. The results are in accordance with data which we published previously regarding the greater toxicity of the cyclic form of aminoalkylisothiourea compared to the corresponding aliphatic form [2].

Both compounds showed a marked radioprotective effect (Table 2). Increase in the compound dose did not result in an increased survival rate of irradiated animals nor in a prolonged protective effect. The value of the radioprotective effect for I and II was observed when they were given 60 min prior to irradiation.

The increase in survival rate of the protected animals is due to the improved state of the hemopoietic system. This is reflected by the increase in spleen weight and in the number of blood-forming cells in the bone marrow of the protected animals (Figs. 1 and 2). By day 15, the spleen weight in these animals was practically no different from that in normal animals. The number of karyocytes in the bone marrow of irradiated animals was significantly higher than in the bone marrow of animals not receiving the protectant, although it did not reach the value which is characteristic for normal nonirradiated animals. A correlation was observed between the survival rate and the degree of recovery of the hemopoietic system. In irradiated, non protected animals there was only a tendency towards normalization of the state of the hemopoietic system. By day 12, all the animals had died.

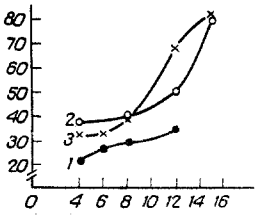


Fig. 1

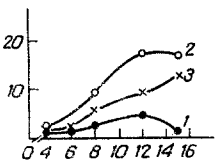


Fig. 2

Fig. 1. Effect of prophylactic administration of I and II on the spleen weight of irradiated mice. 1) Irradiation; 2) I + irradiation; 3) II + irradiation. The time following irradiation (in days) is plotted along the abscissa; the weight of the spleen (in mg) is plotted along the ordinate.

Fig. 2. Effect of prophylactic administration of I and II on the No. of karyocytes in the bone marrow of irradiated mice. 1) Irradiation; 2) I + irradiation; 3) II + irradiation. The time after irradiation is plotted along the abscissa and the No. of karyocytes in bone marrow calculated per $1 \cdot 10^6$ cells of the femur is plotted along the ordinate.

Thus, compounds I and II which we synthesized show a definite radioprotective effect which is related to their ability to decrease radiation damage to the circulatory system and to induce its recovery.

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