

## TRANSFORMATION OF AMINOETHYLISOURONIUM SALTS.

### I. S-AMINOETHYLISOTHIOURONIUM BROMIDE HYDROBROMIDE-<sup>14</sup>C.

L. Kronrad and I. Kozak.

Nuclear Research Institute CSKAE, Rez.  
Biophysical Institute, Charles University,  
Prague - Czechoslovakia.

Received on September 4, 1972.

#### INTRODUCTION

S-aminoethylisothiuronium salts (AET) are considered to be the most efficient radioprotective substances (1,2). They are relatively unstable in aqueous solutions where they are transformed to 2-aminothiazolines (2-AT) (3-6). These substances are more toxic and their radioprotective effect is considerably lower than that of AET (7,8). From this point of view it is important to know the mechanism of the transformation of AET to their derivatives. The kinetics of the formation of 2-AT and mercaptoethylguanidine (MEG) in aqueous solutions of AET has been studied by Bitny-Slach- to (9). The measuring of the optical density of chromatogram spots was used in the case for the determination of the amounts of 2-AT and MEG under various conditions.

The aim of our paper is the determination of the transformation of AET to its derivatives by the help of labelled AET-<sup>14</sup>C.

#### EXPERIMENTAL

##### Materials :

S-aminoethylisothiuronium bromide hydrobromide-<sup>14</sup>C was prepared similarly to the non-radioactive material by means of heating thiourea-<sup>14</sup>C with 2-bromethylamine hydrobromide in isopropyl alcohol at 82°C. The pure crystalline product with m.p. of 192-194°C was obtained by means of its recrystallization from isopropyl alcohol.

Other substances were of A.R. purity; the solvents were dried with fresh heated copper /II/ sulphate and distilled.

#### Transformation of AET-<sup>14</sup>C to 2-AT-<sup>14</sup>C :

0.1 M aqueous solutions of AET-<sup>14</sup>C were heated to 18, 37, 50, 75, and 100°C for various time periods. Samples of reaction mixtures were taken in minute intervals from these solutions and deposited on the chromatographic paper. Because all transformations of substances of the AET type are stopped in strongly acidic media /11/, a drop of concentrated hydrochloric acid was placed beforehand on the chromatogram start.

#### Chromatographic separation :

Samples of the reaction mixture were separated chromatographically on Whatman paper n°3 impregnated by 5 % potassium chloride solution. The butanolic phase of the n-butanol : ethanol (10:1) mixture saturated with 0.2N hydrochloric acid was used as the developing phase in the descendent arrangement. The spots on chromatograms were detected by means of spraying a mixture of nitroprusside with ferrocyanide, described in (9,12). The spots corresponding to AET-<sup>14</sup>C, 2-AT-<sup>14</sup>C, and MEG-<sup>14</sup>C were red - rapidly disappearing with a purple outer edge, permanent blue, and red slowly disappearing, resp.

#### DETERMINATION OF ACTIVITY :

The activity of the chromatograms was determined by a windowless-tube attached to the Frieseke-Hoepfner apparatus.

The percentage of the activity in single chromatographic spots was determined by means of peak area weighing.

#### DATA PROCESSING :

The half-life of the transformation of AET-<sup>14</sup>C to 2-AT-<sup>14</sup>C was calculated from the slopes of the straight lines put through the experimental points by the least square method.

The activation energy was obtained similarly from the dependence of the rate constants on temperature.

#### RESULTS AND DISCUSSION :

S-aminoethylisothiuronium bromide hydrobromide /AET/ is transformed spontaneously in aqueous solutions. This transformation has been described by Doherty et al., who have found 2-aminothiazoline (2-AT) to be the main product(5).

The application of AET labelled by radioactive isotope makes it possible to obtain more detailed information on the course of this process. Paper chromatography of aqueous solutions of AET- $^{14}\text{C}$  has shown that, in addition to spots belonging to AET and 2-AT, radioactive spots belonging to other products of the transformation of AET- $^{14}\text{C}$  can be found on the radiochromatograms.

The  $R_F$  values of all substances resulting from the transformation of AET- $^{14}\text{C}$  are listed in Table 1. Substances with  $R_F = 0.16$ (II) and  $R_F = 0.38$ (III) can be detected only with difficulties by detectors used for colour developing of spots of cyanamide and guanidine derivatives (9). Typical chromatogram of an aqueous solution of AET- $^{14}\text{C}$  demonstrating the presence of all products of transformation is presented in Fig. 1 It was obtained from a sample of an aqueous

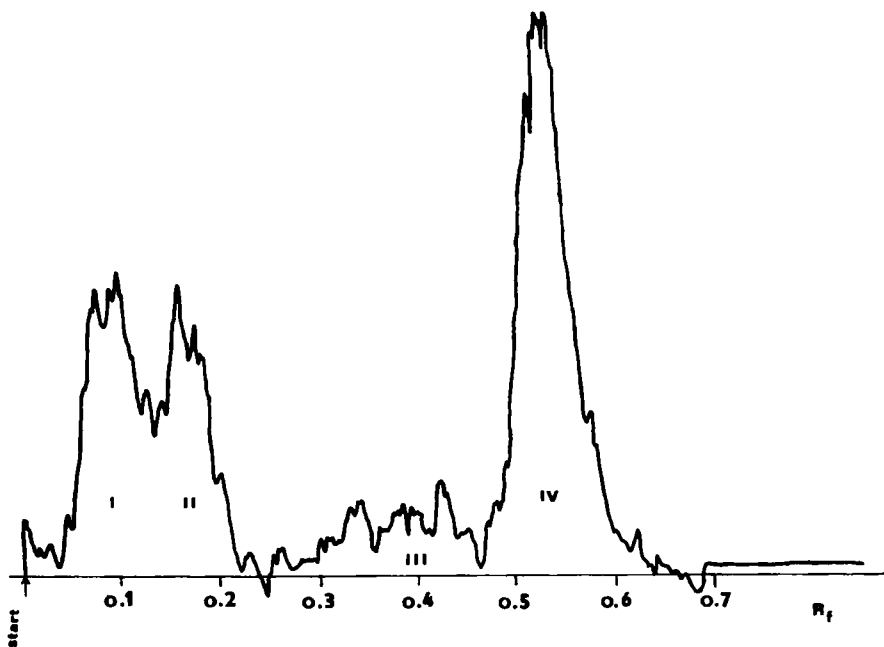


Fig. 1. Radiochromatogram of aqueous solution of AET- $^{14}\text{C}$  after 20 minutes of heating at  $75^\circ\text{C}$ .

Table 1.  $R_F$  values of substances resulting from the transformation of AET- $^{14}\text{C}$  in aqueous solutions.

Substance	I (AET- $^{14}\text{C}$ )	II	III	IV (2-AT- $^{14}\text{C}$ )
$R_F$	0.09	0.16	0.38	0.52

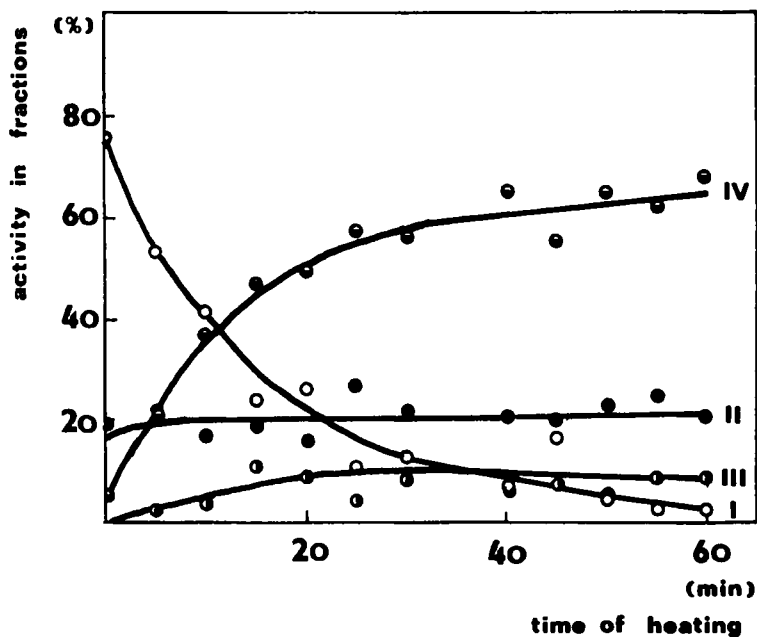


Fig. 2.  $^{14}\text{C}$  activity distribution in percent of total  $^{14}\text{C}$  activity between single products resulting from the transformation of AET- $^{14}\text{C}$  in dependence on time.

solution of AET-<sup>14</sup>C heated to 75°C for 20 minutes. The study of the dependence of the transformation of AET-<sup>14</sup>C on heating time has shown that 2-AT is the main reaction product in all cases. This fact is evident from Fig. 2, where the distribution of the activity of <sup>14</sup>C between AET-<sup>14</sup>C and singular products of its transformation is plotted against time. The percentage of substance II does not practically change with heating time; the percentage of substance III on the other hand slowly increases with increasing heating time.

The fundamental concept on the mechanism of chemical protection against radiation (13) supposes the presence of a free SH group in the radioprotective molecule. 2-AT that has the S atom bound in the cycle both loses the radioprotective properties (14) and becomes more toxic due to its ability to liberate epinephrine from suprarenal gland (15). The detailed study of the transformation of AET-<sup>14</sup>C to 2-AT-<sup>14</sup>C may therefore become an important guide when using aqueous solutions of AET in practice. The rate of this transformation depends considerably on temperature (9). Fig. 3 shows the dependence of the decrease of AET-<sup>14</sup>C content on time of heating of aqueous solutions at 18°C, 37°C, and 50°C,

The linear character of these dependences corroborates the conclusion that the reaction is monomolecular and proceeds according to the following scheme : (13)

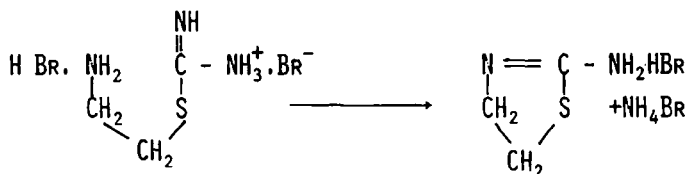


Fig. 4 shows the same dependence as Fig. 3 for temperatures of 75°C and 100°C. Even in this case the character of the dependence remains linear. Reaction rate constants were calculated from the slopes of the straight lines for the single temperatures. The obtained values are given in Table 2; values obtained by bitny-Szlachto (9) for some other temperatures are in good agreement with our results.

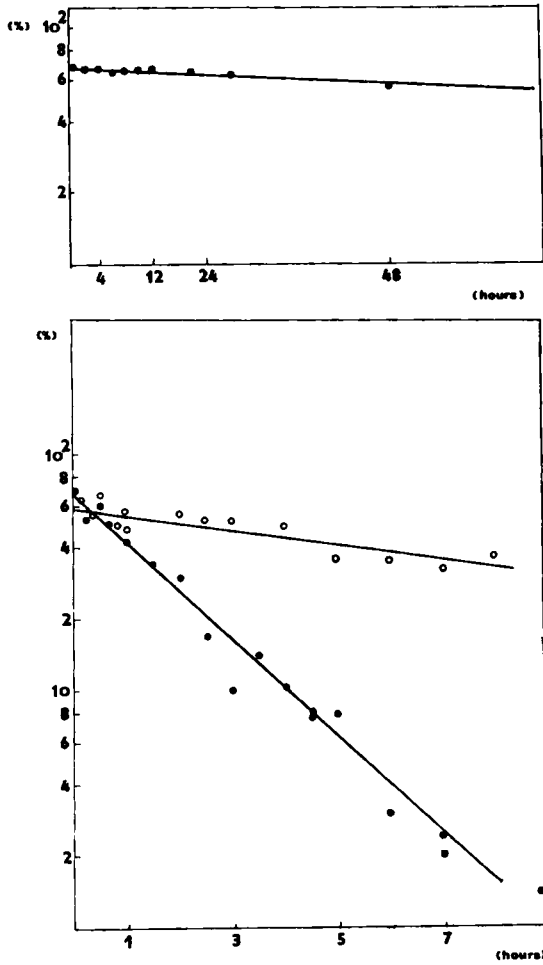


Fig.3 : Dependence of the decrease in AET-<sup>14</sup>C content on time of heating of aqueous solutions  
 a/ 18°C  
 b/ 37°C and 60°C

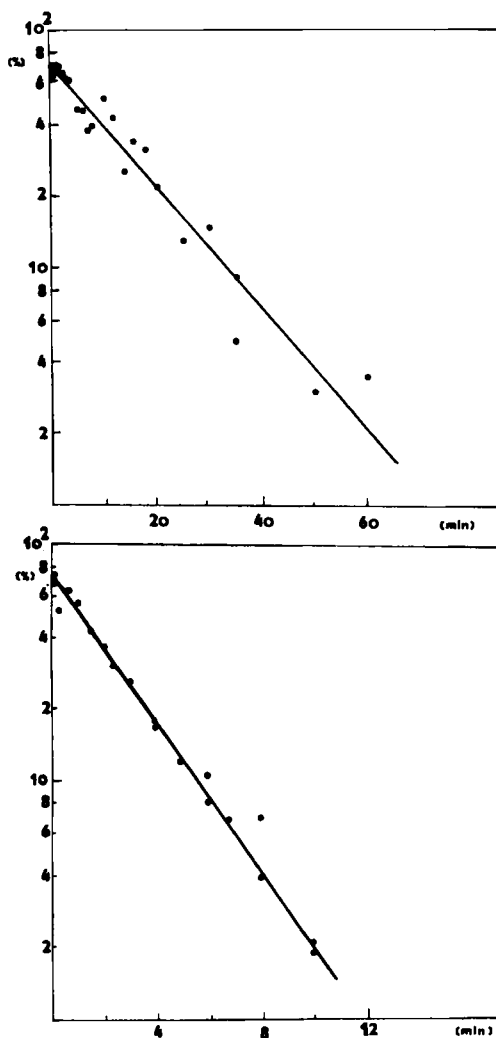


Fig. 4. Dependence of the decrease in AET-<sup>14</sup>C content on time of heating of aqueous solutions.

a) 75°C

b) 100°C

Table 2. Half-life and rate constants of the transformation of AET-<sup>14</sup>C to 2-AT-<sup>14</sup>C at various temperatures.

Temperature	18°C	37°C	50°C	75°C	100°C
k /min <sup>-1</sup> /	69.1 x10 <sup>-5</sup>	11.7 x10 <sup>-4</sup>	78.3 x10 <sup>-4</sup>	58.7 x10 <sup>-3</sup>	37.3 x10 <sup>-2</sup>
T <sub>1/2</sub>	167 hr	9.8 hr	1.5 hr	11.8 min	1.9 min

The dependence of the rate constants on the reciprocal value of absolute temperature is given in Fig. 5. This dependence is also linear and makes it possible to determine the activation energy of this reaction from Arrhenius equation. Substituting values from Fig. 5, the activation energy of 17.5 kcal/mole was obtained. Reciprocal substitution into Arrhenius relation makes it possible to determine the reaction rate for any temperature within the studied interval and by means of an extrapolation even at those temperatures at which the reaction rate is exceedingly low or high. Such an extrapolation yields at 0°C k = 0.29 x 10<sup>-4</sup>/min<sup>-1</sup>, the rate of transformation of AET-<sup>14</sup>C to 2-AT-<sup>14</sup>C at temperatures close to 0°C is very low and therefore even older aqueous solutions of AET may be used in experiments or in practice, if they have been stored at these temperatures.

#### REFERENCES

- 1) DOHERTY, DG., BURNETT, W.T.- Proc. Soc. exp. biol. (NY), 89 : 312 (1955).
- 2) BLOUIN, L.T., OVERMAN, R.R.,-Radiat. Res. 16 : 699 (1962).
- 3) KHYM, J.X., SHAPIRA, R., DOHERTY, D.G.- J. Am. Chem. Soc. 79 : 5663 (1957).
- 4) KHYM, J.K., DOHERTY, D.C., SHAPIRA, R.- J. Am. Chem. Soc. 80 : 3342 (1958).
- 5) DOHERTY, D.G., SHAPIRA, R. BURNETT, W.T. - J. Am. Chem. Soc. 79 : 5667 (1957).
- 6) SHAPIRA, R., DOHERTY, D.G., BURNETT, W.T. - Radiat. Res. 7 : 22 (1957).
- 7) SHASHKOV, V.S., FEDOSEEV, V.M. - Med. Radiol. 6 : 25 (1961).



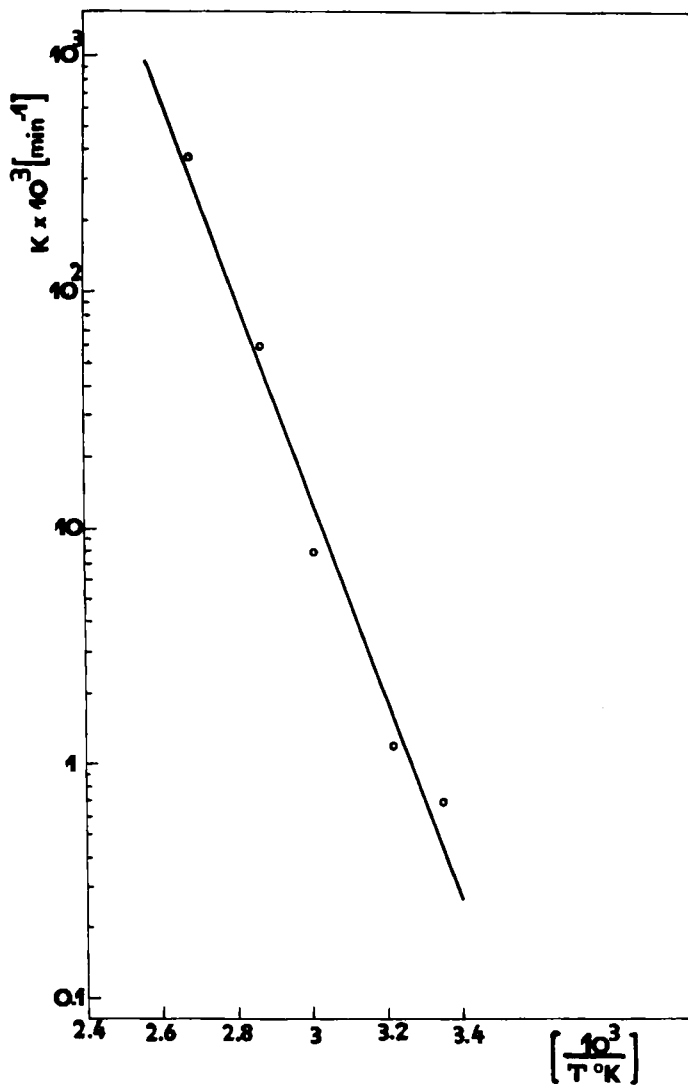


Fig. 5. Dependence of rate constants on absolute temperature.

- 8) KOZAK, I., ARIENT, M.- to be published
- 9) BITNY-SZLACHTO, S.- Acta Poloniae Pharmaceutica 25 : 411 (1968).
- 10) CLINTON, P.O., SABOTA, U.J. - J.Am. Chem. Soc. 70 : 910 (1948).
- 11) SHAPIRO, B., SCHWARTZ, E.E., KOLLMAN, G. - Cancer Res. 23 : 223 (1963).
- 12) WALDI, D., Sprühreagentien für die Dünnschicht-Chromatographie, in  
Dünnschichtchromatographie, ed. E. Stahl,  
p. 510, Springer Verlag, Berlin 1962.
- 13) BROWN, P.E.-Nature 213 : 363 (1967).
- 14) DISTEFANO, V., LEARY, D.E., LITTLE, K.D. - J. Pharmacol. exp.  
Ther., 126 : 158 (1959).
- 15) ARIENT, M., KOURILEK, K., Pospisil, J., DIENSTBIER, Z., KYRAL, V.-  
Cas. lék. českých 108 : 1470 (1969).