

TRANSFORMATIONS OF AMINOETHYLISOURONIUM SALTS.

II. SE-AMINOETHYLISOSELENOURIUM BROMIDE HYDROBROMIDE-⁷⁵Se.

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INTRODUCTION

Thiourea and its derivatives, especially S-aminoethylisothiouronium salts, have been found to be important radioprotective substances(1). Similar results were also obtained with some selenium compounds, especially with selenourea (2-4). Detailed study of the radioprotective substances have shown that the most suitable substances are those that possess a basic functional group in beta-position with respect to sulphur. Se-aminoethylisoselenouronium bromide hydrobromide may be therefore expected to possess also radioprotective properties.

S-aminoethylisothiouronium bromide hydrobromide (AET) has been proved on the other hand to accumulate considerably in the tissue of a sound heart. From this point of view the Se analog of this substance labelled with radioactive gamma-emitter could be suitable for scintigraphic detection of ischaemic heart disease.

The most significant chemical property of AET is its transformation in aqueous solutions to 2-aminothiazoline. We have tried therefore to prepare Se-aminoethylisoselenouronium bromide hydrobromide-⁷⁵Se and to determine its behaviour in aqueous solutions.

EXPERIMENTAL

Materials :

Se-aminoethylisoselenouronium bromide hydrobromide-⁷⁵Se (AE-⁷⁵Se) was prepared by multistage synthesis in the following way :

Selenium (IV) oxide- ^{75}Se prepared by means of neutron irradiation in VVR-S nuclear reactor in Rez, Czechoslovakia, was reduced according to Stejskal, Kronrad and Cifka (6) to selenium hydride- ^{75}Se . Its absorption in ether solution of ammonia and cyanamide (7) led to the formation of selenourea- ^{75}Se . AE^{75}Se was prepared in a way similar to that applied for the preparation of inactive sample (8) by means of condensation of selenourea- ^{75}Se with 2-aminoethyl bromide hydrobromide in isopropyl alcohol medium. Its recrystallization from ethanol - ethyl acetate 1:1, v/v mixture yielded pure product with m.p. of 204-206°C.

2-aminoselenazoline- ^{75}Se ($2\text{-A}^{75}\text{Se}$), used as the chromatographic standard, was prepared by means of the dissolution of AE^{75}Se in water; the solution was refluxed for 30 minutes with simultaneous bubbling through of gaseous carbon dioxide followed by vacuum evaporation to dryness. The pure product with m.p. of 170°C was recrystallized from ethyl alcohol. Analytically determined content of selenium corresponded to the calculated one.

All remaining reagents were of A.R. purity, ethyl alcohol, ethyl acetate, and isopropyl alcohol were dried by fresh heated copper sulphate and distilled.

METHODS :

The study of the kinetics of the transformation of AE^{75}Se to $2\text{-A}^{75}\text{Se}$, chromatographic separation of the mixtures of these substances and the processing of the results was similar to that described in the preceding paper. The spots on chromatograms were colour developed by means of spraying a mixture of 5 ml of 10% solution of potassium ferrocyanide, 5 ml of 10% sodium nitroprusside, and 5 ml of 10% sodium hydroxide diluted with 90 ml of distilled water and 120 ml of acetone. The spots corresponding to AE^{75}Se , $2\text{-A}^{75}\text{Se}$, and selenomerkaptoethyl guanidine- ^{75}Se (MEG^{75}Se) were red, rapidly disappearing, permanent blue, and red slowly disappearing, resp.

RESULTS AND DISCUSSION

Considerable chemical similarity between selenium and sulphur enables to suppose that similarly to sulphur derivatives (9) AE^{75}Se is transformed to $2\text{-A}^{75}\text{Se}$. Preliminary experiments corroborates this supposition and indicated that in the case of AE^{75}Se a series of transformation products is

also formed in aqueous solutions. A typical chromatogram of the aqueous solution of AE^{75}Se on which all transformation products are present is presented in Fig. 1.

The chromatogram was obtained from a sample of 0.1 M aqueous solution of AE^{75}Se heated to 75°C for 15 minutes. Fig. 1. shows that in addition to $2\text{-A}^{75}\text{Se}$,

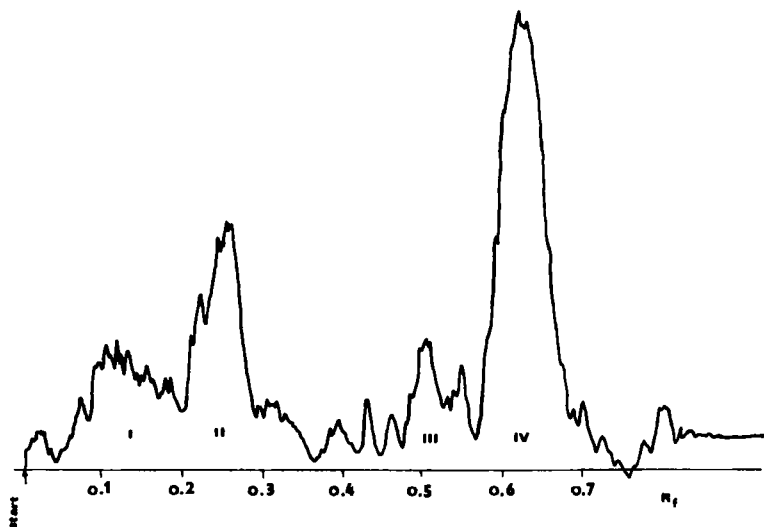


Fig.1 : Radiochromatogram of aqueous solution of $\text{AESe}^{75}\text{Se}$ after 20 minutes of heating at 75°C .

which is the main transformation product, two more substances may be found on the chromatograms. R_f values of all of these substances in the system n-butanol : ethanol (10:1) saturated with 0.2 N hydrochloric acid solution are given in Table 1. The comparison of both the chromatograms and the R_f values with the cor-

Table 1. R_f values of substances resulting from the transformations of $\text{AESe}^{75}\text{Se}$ in aqueous solutions.

Substance	I ($\text{AESe}^{75}\text{Se}$)	II	III	IV ($2\text{-ASe}^{75}\text{Se}$)
R_f	0.10	0.19	0.40	0.55

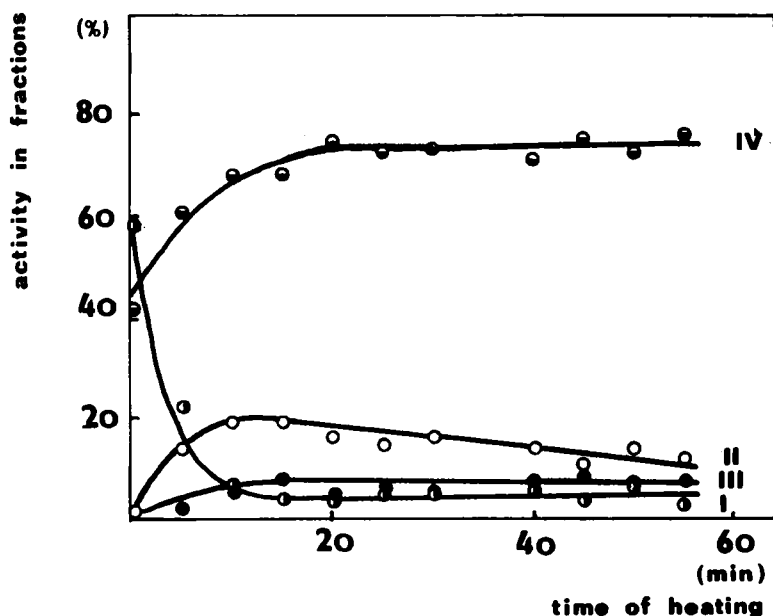


Fig.2 : ^{75}Se activity distribution in percent of total ^{75}Se activity between single products resulting from the transformation of $\text{AESe-}^{75}\text{Se}$ in dependence on time.

responding results obtained for the transformation of $\text{AET-}^{14}\text{C}$ (10) show that AE^{75}Se does not practically differ in the basic transformation scheme in aqueous solutions from $\text{AET-}^{14}\text{C}$. The colours of the spots on chromatograms after spraying the detection agent were also identical and the stability of the colouring was comparable.

The dependence of the percent amounts of the transformation products of AE^{75}Se on the time of heating is given in Fig.2. The largest increase in the percentage of activity can be observed with 2-A ^{75}Se , the percentage of the activity in the spot with $R_F=0.19$ (II) increase at the beginning and after 15 minutes of heating it slowly decreases. The amount of the substance with $R_F=0.40$ (III) slowly increases at the beginning and after 15 minutes of heating an equilibrium is established. In the contradistinction to the similar dependence for $\text{AET-}^{14}\text{C}$ when the substance with $R_F=0.16$ could be found even at the beginning i.e. it is formed during the preparation of $\text{AET-}^{14}\text{C}$, in the case of AE^{75}Se , it is formed only during heating in the aqueous solution. This fact is clearly evident also

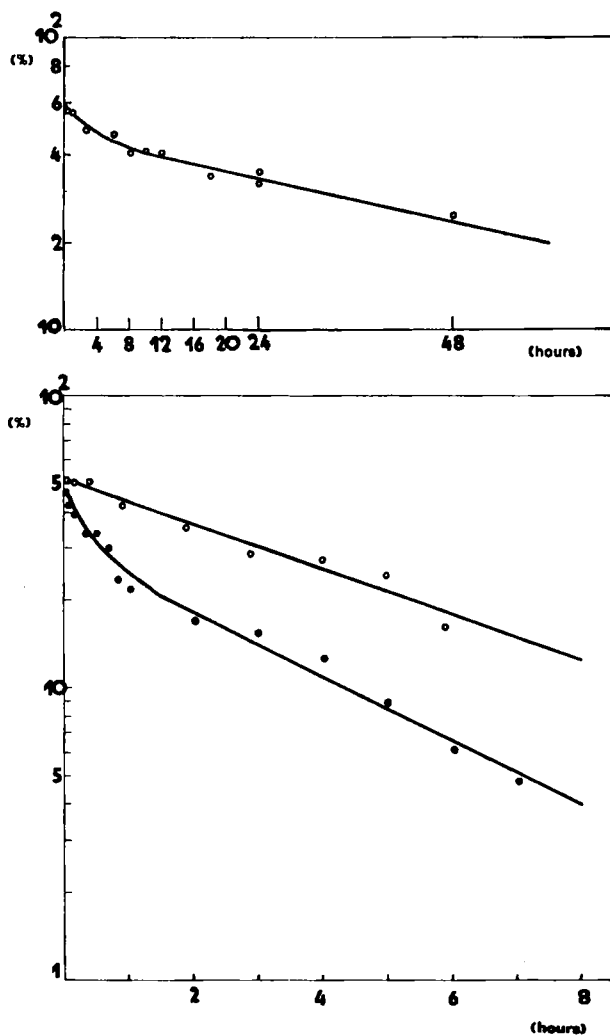


Fig.3 : Dependence of the decrease in AESe-⁷⁵Se content on time of heating of aqueous solutions
 a/ 18°C
 b/ 37°C and 50°C

in Fig.3, where the dependance of the decrease of the content of $AE^{75}Se$ on the time of heating of its aqueous solutions to 18°C, 37°C, and 50°C is shown. The obtained experimental curves have a non-linear character and their graphical analysis yielded two straight lines corresponding to different reactions. The straight line with the smaller slope corresponds probably to the transformation of $AE^{75}Se$ to $2-A^{75}Se$, that with the larger slope to the transformation of $AE^{75}Se$ to substances II and III. With respect to the fact that the dependence corresponding to the transformation of $AE^{75}Se$ to $2-A^{75}Se$ is linear, monomolecular reaction according to the scheme.

Table 2. Half-life and rate constants of the transformation of $AESe^{75}Se$ to $2-ASe^{75}Se$ at various temperatures.

Temperature	18°C	37°C	50°C	75°C	100°C
$k \text{ /min}^{-1} /$	69.1 $\times 10^{-5}$	11.7 $\times 10^{-4}$	78.3 $\times 10^{-4}$	58.7 $\times 10^{-3}$	37.3 $\times 10^{-2}$
$T_{1/2}$	167 hr	9.8 hr	1.5 hr	11.8 min	1.9 min

and similar to that with $AET^{14}C$ may be supposed. Similar dependences for the temperatures of 75°C and 100°C are presented in Fig.4. Two straight lines corresponding to the transition of $AE^{75}Se$ to $2-A^{75}Se$ and to substances II and III could be also found for these temperatures. Reaction rate constants were therefore calculated for the transition of $AE^{75}Se$ to $2-A^{75}Se$ from the slopes of the straight lines at various temperatures. The obtained values of the rate constants are listed in Table 2. These values are evidently lower when compared with the corresponding values obtained with $AET^{14}C$.

The dependence of the rate constants on reciprocal absolute temperature is shown in Fig.5. The linear character of this dependence makes it possible to determine the activation energy from Arrhenius equation. The obtained value of 16.3 kcal/mole leads to the conclusion that the transformation of $AE^{75}Se$ to $2-A^{75}Se$ is in practice negligible when the aqueous solutions are stored at temperatures close to 0°C.

In general it may be stated that the transformation of $AE^{75}Se$ and $AET^{14}C$ yields practically similar products, the dominating transformation being in both cases the transformation to $2-A^{75}Se$ and $2-AT^{14}C$, resp.; the rate of the transformation of $AE^{75}Se$ is slightly lower.

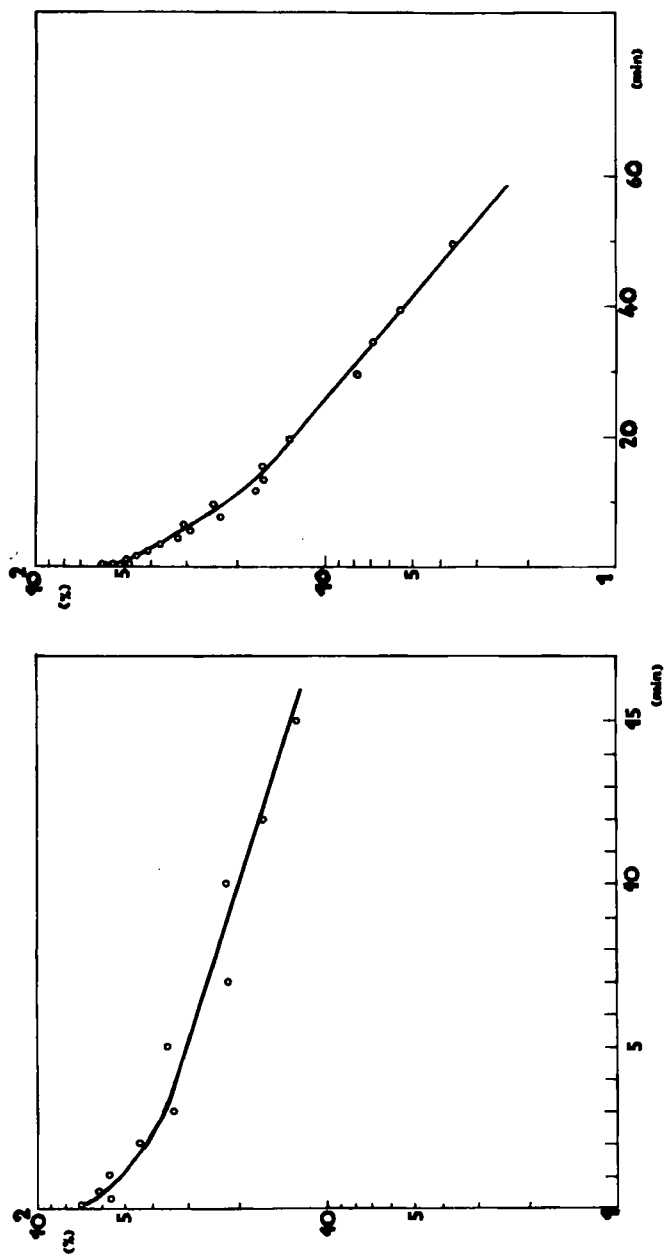


Fig. 4 : Dependence of the decrease in AESe-⁷⁵Se content on time of heating of aqueous solutions
a/ 75°C
b/ 100°C

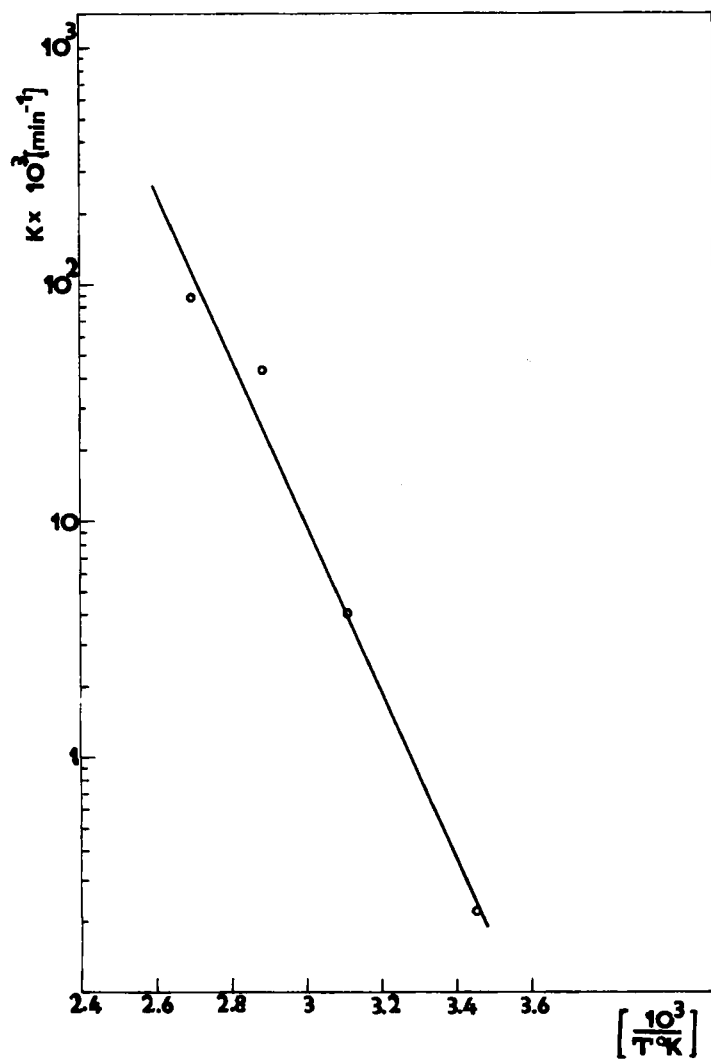


Fig.5 : Dependence of rate constants on absolute temperature.

Because chemical behaviour of both substances is very similar, their behaviour in an organism, mainly as far as the metabolism is concerned, may be expected to be similar, too.

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