Journal of Labelled Compounds — October-December — Vol. VI, No. 4

Preparation of some mercuri-derivatives of fluorescein labelled with isotopes ^{197}Hg or ^{203}Hg.

II. Study of conditions for optimum yield of individual fractions

L. Kronrád, J. Ratuský, P. Málek, B. Vavrejn and J. Kolc.

Nuclear Research Institute, Institute of Organic Chemistry and Biochemistry. Institute of Clinical and Experimental Surgery. Research Institute for Medical Use of Radioisotopes in Medicine.

Received January 13th 1970.

Summary

The work is a continuation of the preceding study of possibilities for preparation of labelled derivatives of fluorescein. The conditions under which it is possible to get maximum possible yield of individual products from reaction mixture of mercuric acetate-^{203}*Hg and fluorescein has been studied in detail. It has been found that the yield of derivatives with different mercury content depends to a great extent on the molar ratio of both reaction components. In the first, rapid step of mercurization approximately the same amount of all products is formed regardless of the molar ratio of reaction components. By heating the equilibrium is shifted in favour of the product corresponding to the given molar composition of reaction mixture. The detailed procedures for preparation of difluoresceinyl mercury-*^{203}*Hg and hydroxymercurifluorescein-*^{203}*Hg have been given.*

Introduction.

In our previous work (1) dealing with study of possibilities of preparation of mercuri-derivatives of fluorescein it was showed, that exchange reaction, which is very fast has a disadvantage namely the reaction is accompanied by mercurization and therefore the yield of fractions mercurized to higher degrees is increased.

Individual fractions separated chromatographically from mixture of mercuri-derivatives of fluorescein after the substitution reaction differ after the administration into the organism by difference in the rate of clearance and by the difference in the cumulation in the damaged and in normal tissues (3). Most convenient properties in this respect were found for fractions with lower content of mercury. Therefore, preparations for clinical use were prepared by normal substitution reaction. These fractions, however are formed with rather low yields; we tried therefore to find optimum conditions for their preparation in higher yields.

EXPERIMENTAL.

Mercuric acetate-^{203}Hg and chromatographically pure fluorescein were prepared in the same way as in our previous work (1).

Observation of the course of mercurization reaction.

Aqueous solution of chromatographically pure sodium salt of fluorescein was mixed with mercuric acetate-^{203}Hg accompanied by formation of a precipitate. The reaction mixture was heated under reflux for 1 hour. Immediately after mixing in cold first sample was withdrawn for chromatographic separation. In short intervals samples were taken out of boiling mixture and after dissolution by addition of one drop of 0.1 N sodium hydroxide were applied on the chromatographic paper. Activity was measured after development of chromatograms. Withdrawn samples were applied also on the thin-layer of alumina to measure the content of unreacted ionic mercury in the reaction mixture. The influence of pH on the distribution of products was studied by heating the reaction mixture in 0.2 mole acetate buffer.

Separation of products.

Paper-chromatography was carried out in descending arrangement on Whatman paper No. 3 in mixture of 6 % of ammonia-methanol (1 : 1).

The content of unreacted ionic mercury was determined on alumina by thin-layer chromatography in 1 % aqueous solution of acetic acid.

Counting.

Radioactivity was counted by means of an ACEC apparatus with a well-type crystal.

RESULTS AND DISCUSSION.

The study of conditions for maximum yield of Mercurascan was carried out using a reaction mixture with ionic mercury labelled with ^{203}Hg. With respect to the fact that the mercurization of fluoresceine can proceed to second stage, it was interesting to study the distribution of products as a function of the concentration ratio of fluorescein and ionic mercury. The yields of individual products after 60 minutes heating (i.e. after the equilibrium was reached) as function of molar fraction of reacting components are shown

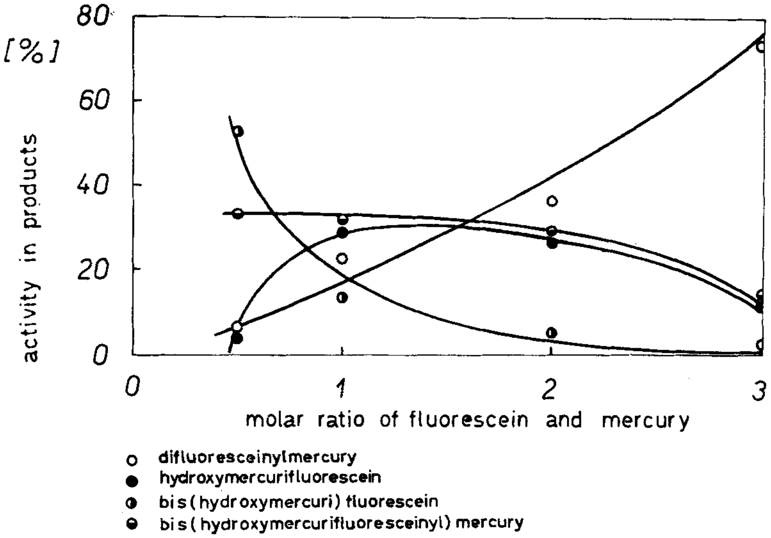

FIG. 1. Yiels of individual products of mercurization of fluorescein in dependence on the molar ratio of the fluorescein and mercury.

in Figure 1. From this figure one can see that the concentration of fraction IV corresponding to *bis* (hydroxymercuri)fluorescein increases in reaction mixture with an excess of ionic mercury. On the contrary in mixture with an excess of fluorescein most of activity is found in a spot corresponding to difluoresceinylmercury. The yield of *bis* (hydroxymercurifluoresceinyl)mercury is also lowered by excess of fluorescein and hydroxymercurifluorescein is formed with greatest yield at a molar ratio of fluorescein and ionic mercury 1 : 1. The figure also shows that in all cases (with excess of fluorescein as well as with excess of ionic mercury) all products are formed. This means that in all cases the product after the preparation must be purified by some of the chromatographic methods. It is convenient to use an excess of fluorescein if we wish to prepare compounds with lower mercury-content (Mercurascan).

The curves representing the course of mercurization in dependence on the time of heating for given molar ratio of fluorescein and ionic mercury are presented in Figure 2. Figures 2*a*, 2*b*, 2*c* and 2*d* refer to the ratio 1 : 2, 1 : 1, 2 : 1 and 3 : 1 respectively.

Polarographic determination of the decrease of the concentration of ionic mercury in the reaction mixture has shown that the initial stage of mercurization is very fast (1). Figure 2*a*, *b*, *c* and *d* show that after fast formation of a bond between mercury and fluorescein, a second slower stage of the reaction takes place, where the interaction between individual mercuri-derivatives of fluorescein occurs. It is interesting that in initial stage of the reaction the molar ratio of fluorescein and mercury in the reaction mixture

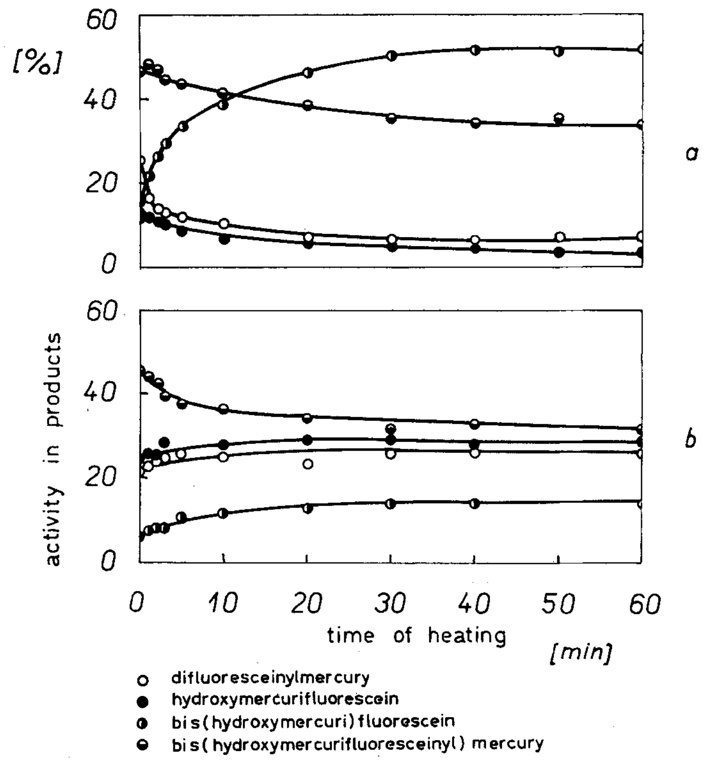

FIG. 2. Yields of individual products of mercurization of fluorescein in dependence of the time of heating :

(*a*) the molar ratio of fluorescein and mercury 0.5 : 1.

(*b*) the molar ratio of fluorescein and mercury 1 : 1.

does not play any significant role (the same products with the same yields within a few of per cent are formed) and the difference becomes more pronounced only after the subsequent reactions of individual substances between each other took place.

The dependence of yields of individual substances on the pH (after heating for 60 minutes) are shown in Figure 3. From this Figure one can see that there is practically no difference in the distribution of products within the range of pH values 3.6-5.0. These experiments also showed that the ratio of yields of individual fractions at constant pH varies with the time of heating. These changes, however, are the same for various pH values. A typical course of mercurization as a function of the time of heating at pH 4.4 is shown in Figure 4.

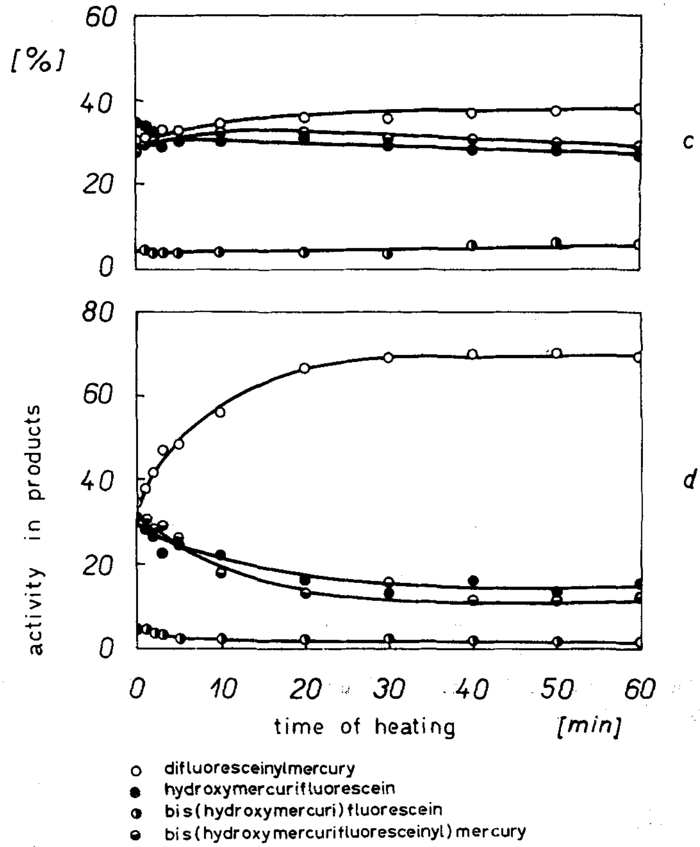

FIG. 2 *(suite)*
(*c*) the molar ratio of fluorescein and mercury 2 : 1.
(*d*) the molar ratio of fluorescein and mercury 3 : 1.

The results and experience led us to the following procedure for preparation of mercuri-derivatives of fluorescein labelled with ^{197}Hg or ^{203}Hg :

50 mg of mercuric oxide-^{203}Hg is dissolved in 0.2 ml of conc. acetic acid diluted with 2 ml of distilled water. 5 ml of 0.1 N acetic acid and 5 ml of 0.1 N sodium acetate are added.

227 mg of chromatographically pure fluorescein is dissolved in 8.5 ml of 0.1 N sodium hydroxide and the solution is added to the solution of mercuric acetate-^{203}Hg. The reaction mixture is heated to boiling temperature for 1 hour. After heating the mixture is dissolved in 10.5 ml of 0.1 N sodium hydroxide and the pH is adjusted to 7.5 by dilute acetic acid. So treated solution is transferred on the alumina column (the diameter and the lenght of the column being 2 cm and 40 cm respectively) of the lenght of 15 cm, prepared by stirring of 60 g of dry alumina in distilled water. After soaking of

The described method of preparation yields difluoresceinylmercury with a yield of 60-70 %

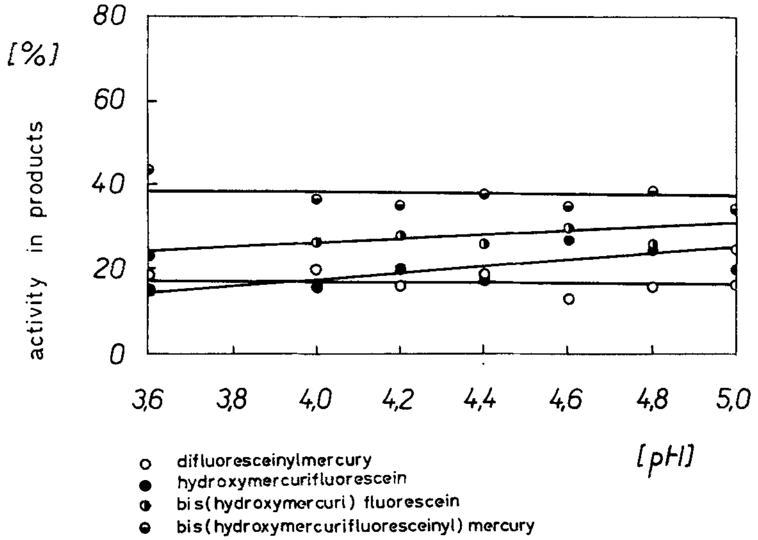

FIG. 3. The dependence of yields of individual products on the pH for equimolar mixture of fluorescein and mercury.

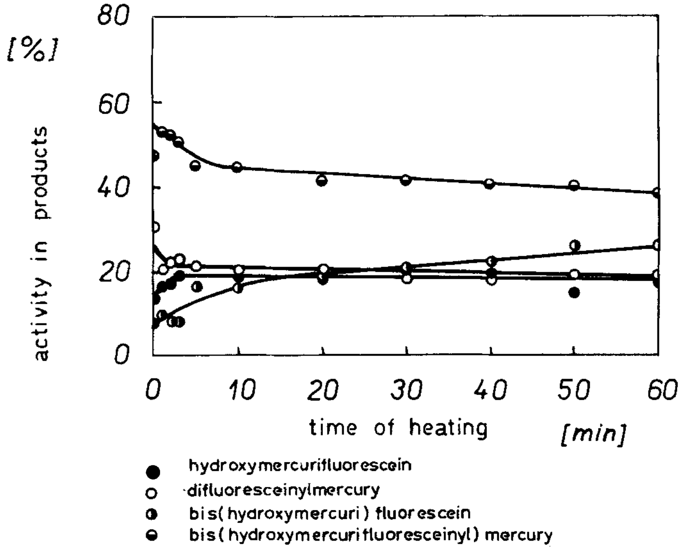

FIG. 4. Yields of individual products in dependence on the time of heating (the pH = 4.4; equimolar mixture of fluorescein and mercury.

the solution into the column it is eluted by 0.02 sodium bicarbonate. About 120 ml of eluate is required for elution of unreacted fluorescein. Difluoresceinylmercury is eluted by additional 20-30 ml of eluate. The pH of eluate is adjusted to 7.0-7.5 by addition of 1-2 ml of 0.1 N acetic acid. The overall time required (without chromatographic control) is about 8 hours.

This method can be used especially for preparation of «Mercurascan» labelled with longer lived ^{203}Hg. For the preparation of this compound labelled with ^{197}Hg the direct neutron irradiation proved to be more convenient as described in our next communication in this series.

REFERENCES

1. Ratuský, J. Kronrád, L. Málek, P., Vavrejn, B. and Kolc, J. — This Journal.
2. Málek, P., Ratuský, J., Vavrejn B., Kronrád, L. and Kolc, J. — *J. Nucl. Med.* (to be published).