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

I. Study of preparation methods.

J. RATUSKÝ, L. KRONRÁD, P. MÁLEK, B. VAVREJN and J. KOLC.

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

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SUMMARY

A method for preparation of mercuri-derivatives of fluorescein labelled by ^{197}Hg or ^{203}Hg from mercuric acetate and fluorescein has been described. Individual products formed in the reaction mixture were isolated with both paper chromatography and chromatography on alumina column. Their identification was carried out by determining the amount of mercury contained in individual chromatographic fractions. Polarographic observation of the overall mercurization reaction has shown that proceeds rather fast. Similar situation is also valid for exchange reaction between mercuric-acetate-^{203}Hg and individual derivatives of fluorescein.

INTRODUCTION

Organic compounds labelled with ^{197}Hg or ^{203}Hg are more and more frequently used in medicine for diagnostic purposes. Some iodinated derivatives of tetracycline labelled with ^{131}I and mercuric-derivatives of fluoresceine labelled with ^{197}Hg or ^{203}Hg were prepared by Málek and coauthors [1,2] during the study of the incorporation of various substances into the damaged tissues. Some of these substances accumulate to a great extent in damaged tissues. Substances with most convenient properties such as fast clearance of blood, large difference between the cumulation in the normal and injured muscles etc. were utilised for elaboration of a method for detection of heart-attack *in vivo* [3].

Organic labelled compounds can generally be prepared in three ways: (i) by synthesis (ii) by exchange reactions (iii) by direct neutron irradiation.

Nagase and Ohno [4] dealt with the synthesis of inactive mercuric-derivatives of fluorescein and found out that these compounds can be easily prepared by a simple substitution reaction of mercuric-acetate and fluorescein. By chromatographic analysis of the product two other substances were found besides unreacted fluorescein [5,6].

Exchange reactions between inorganic and organic compounds of mercury were described by some authors who studied chemical effects of nuclear transformations in some organic compounds of mercury [7,8].

The preparation of some medicinal compounds of mercury (chlormerodrin, merallurid and BMHP) by exchange reaction was described by Cífka, Kronrád and Kačena [9]. The exchange reaction between ionic mercury and organic compounds goes in these cases rather slowly; however, it is much simpler than the preparation by synthesis.

The preparation of chlormerodrine-^{197}Hg and meralluride-^{197}Hg by irradiation with neutrons was studied by Kronrád and Cífka [10]. They found it possible to prepare these compounds with a specific activity up to 40 mCi/g.

In the present work we attempted to prepare mercuric-derivatives of fluorescein labelled with ^{197}Hg and ^{203}Hg and to isolate and identify the individual products of the mercurization reaction.

Experimental

Chemicals.

Reagent grade fluorescein (a product of Lachema) was purified chromatographically on the alumina column. Distilled water was used as the eluant; only the first coloured fraction of the three present on the column was collected.

Mercuric oxide-^{197}Hg and ^{203}Hg was prepared by irradiation of nonradioactive oxide by neutrons in the VVR-S reactor of The Nuclear Research Institute (of Czech. Acad. Sci.) at Rez. The specific activity reached was of the order of 1,000 mCi/g and 140 mCi/g respectively. Mercuric acetate-^{197}Hg and ^{203}Hg was prepared by dissolving the mercuric oxide-^{197}Hg and ^{203}Hg in diluted acetic acid and by careful evaporation the solution to dryness.

Aluminium oxide according to Brockman used for column chromatography was a product of Reanal (Budapest). All other used chemicals were of reagent grade purity.

Observation of the overall course of the mercurization reaction.

Equimolar mixture of aqueous solution of sodium salt of fluorescein (1 mg/ml) and mercuric acetate (1 mg/ml) was allowed to stand in cold. Samples were withdrawn at appropriate time intervals and subjected to polarographic analysis for inorganic mercury. The same procedure was used in case of hydroxymercuri-fluoresceine and *bis* (hydroxymercuri)fluoresceine.

The separation of products of mercurization reaction.

Equimolar mixture of the sodium salt of fluorescein and of mercuric acetate in aqueous solution was heated under reflux for 1 hour. The product was separated on the alumina column. Solutions of 0.02 mole $NaHCO_3$ and 0.1 N NaOH were used as eluants. First two fractions (I and II) were eluted by the former and next two fractions (III and IV) by the latter. The purity of separated fractions was tested by paper-chromatography on Whatman No. 2 in ascending arrangement with the mixture methanol —6 % of ammonia (1 : 1).

Observation of the course of exchange reaction.

Solutions of *bis* (hydroxymercuri) fluorescein and of mercuric acetate-^{197}Hg were applied immediately one after another on the same spot of thin-layer of alumina. The chromatogram was then developed by dilute acetic acid, where the ion Hg^{2+} goes with the front. The time interval between the application of the second reaction component and the time when the front of the chromatographic mixture reached the spot was taken as the time of contact of both reactants. To test the correctness of this procedure it was modified so that mercuric acetate-^{197}Hg and *bis* (hydroxymercuri) fluorescein were applied separately on two different spots, mercuric acetate being applied nearer to the start. The interval between the time when the front reached the spot of *bis* (hydroxymercuri) fluorescein and the time when it fully left it was taken as the time of contact.

Determination of mercury in products.

Mercuric acetate-^{203}Hg of exactly known specific activity was used to prepare the reaction mixture, which was applied on the Whatman paper No. 3 and chromatographed. The individual spots were cut out and eluted by distilled water. The activity of eluates was measured under geometry identical with measurement of stock solution of mercuric acetate-^{203}Hg. The concentration of products in eluates was determined spectrophotometrically. The chemical content of mercury was calculated from the ratio of specific activities in individual fractions to the specific activity of the stock solution of mercuric acetate-^{203}Hg.

Counting.

Counting of chromatograms and of eluted fractions was made under constant geometry with a well-type scintillation crystal in an apparatus ACEC. The radiochemical purity of the starting mercuric oxide-^{197}Hg was tested by measurement of the gamma-spectra by means of a 200 channel pulse-height analyser Intertechnique.

RESULTS AND DISCUSSION.

The mercurization of fluorescein and its derivatives leads to a great numbers of substances. The separation on the alumina column as well as by paper-chromatography or by thin-layer chromatography was tried. For separation of small quantities, chromatography on Whatman No. 3 paper with the mixture methanol-6 % of ammonia (1 : 1) proved to be the most convenient. The R_f values of all products of mercurization of pure fluorescein are given in Tab. 1. Individual fractions in this case correspond to those isolated by subsequent elution out of the column. More detailled paper-chromatography showed that fraction II consists of four other substances with very close R_f values. However, if the travelled distance is long enough (about 50 cm) all 7 derivatives can be separated in a single chromatogram.

TABLE 1. R_f-values of individual fractions in the mercurization mixture of fluorescein.

Fraction	I	II		III	IV
R_f	0.57	0.43	0.31	0.17	0.03

The activity distribution among the fractions I to IV is shown in Figure 1. This distribution refers to an equimolar mixture of fluorescein and mercuric acetate-^{197}Hg and indicates that in this case all derivatives are formed simultaneously and with relatively uniform abundance.

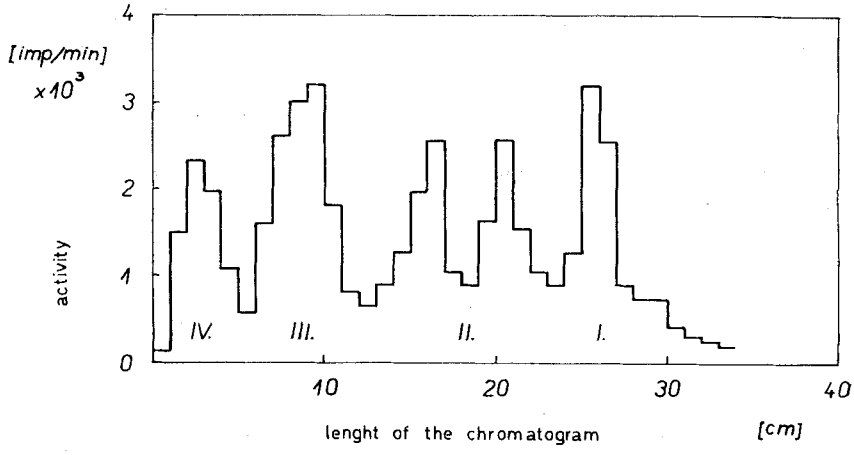

FIG. 1. The activity distribution among individual fraction in the reaction mixture of fluorescein and mercuric acetate-^{203}Hg.

The chemical content of mercury in individual chromatographic fractions is given in Table 2. Fraction I corresponds by the chemical composition roughly to difluoresceinylmercury, fractions II correspond to hydroxymercurifluorescein (the content of mercury was determined only for three of four components of this fraction), fraction III corresponds to *bis* (hydroxymercurifluoresceinyl) mercury and fraction IV to *bis* (hydroxymercuri) fluorescein.

TABLE 2. Chemical content of mercury in individual fractions.

Fraction	I	II		III	IV
Theory	23.5	36.8		46.7	52.7
Analysis	21.0	33.4	35.4	45.9	52.4

The overall rate of mercurization reaction was followed polarographically by determination of the decrease of ionic mercury content. The first curve shows that overall mercurization of fluorescein is rather fast even in cold and is pratically finished in 30 minutes. In the second case there is practically no decrease of ionic mercury content; the mercurization to the 3rd stage therefore does not take place.

The results of exchange reaction between mercuric acetate-^{203}Hg and *bis* (hydroxymercuri)fluorescein are given in Figure 3. Practically the same result are gained also for exchange reaction between mercuric acetate-^{203}Hg and hydroxymercuri-fluorescein.

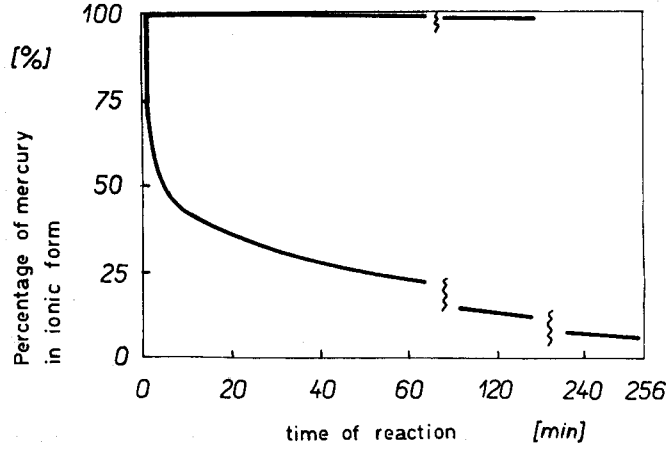

FIG. 2. Decrease of ionic-mercury content in the reaction mixture as a function of time.
(*a*) reaction mixture of fluorescein and mercuric acetate-^{203}Hg.
(*b*) reaction mixture of *bis*(hydroxymercuri) fluorescein and mercuric acetate-^{203}Hg.

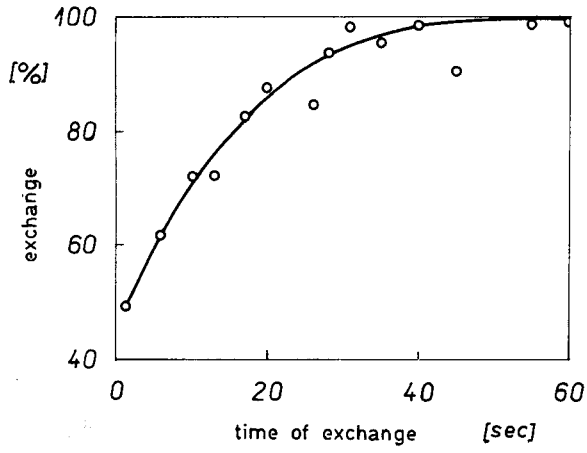

FIG. 3. The time dependence of the exchange reaction between *bis*(hydroxymercuri) fluorescein and mercuric acetate-^{203}Hg.

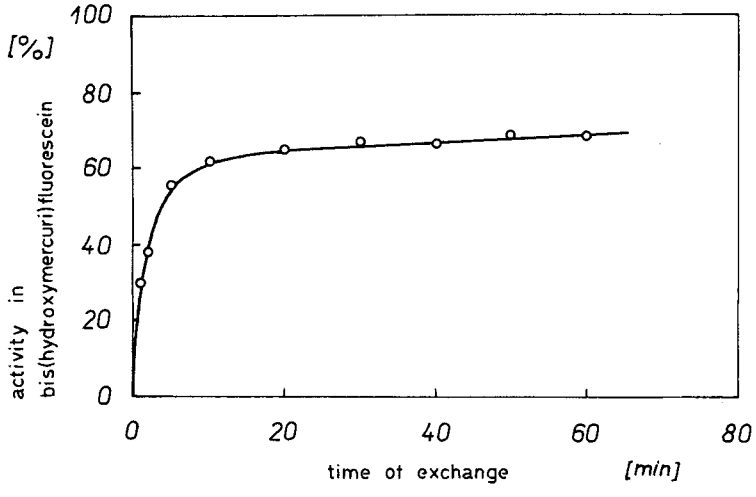

FIG. 4. The time dependence of the exchange reaction between hydroxymercurifluorescein-^{203}Hg and *bis* (hydroxymercuri) fluorescein.

The fast course of the exchange reaction might allow an elegant method of preparation of labelled mercuri-derivatives of fluorescein. However, because the mercurization reaction is also fast, the fraction of components mercurized to higher degree may be enlarged due to the competition of the two mentioned reactions. Exchange reaction can be therefore safely used only for preparation of *bis* (hydroxymercuri) fluorescein, where there is no more mercurization (see Fig. 2).

The rate of exchange between individual fractions in the reaction mixture was also studied. It turned out that at room temperature there is practically no exchange between individual fractions, while at 100° C the transfer of activity from one fraction to another is completed within 20 min. (see Fig. 4).

As various mercuri-derivatives of fluorescein may differ in their behaviour in a living organism and may have also different toxicities, we have studied the behaviour of individual fractions separately and we concluded that most convenient for topic diagnostic of the infarction of myocardium are the compounds with lower mercury content.

In connection with this we have to say that fraction with the lowest mercury content (difluoresceinylmercury) is an unstable compound and transforms to hydroxymercurifluorescein. The time-dependence of the decomposition of difluoresceinylmercury is shown in Figure 5.

As both compounds (decomposing difluoresceinylmercury and forming hydroxymercurifluorescein) behave in the organism alike, their equilibrium

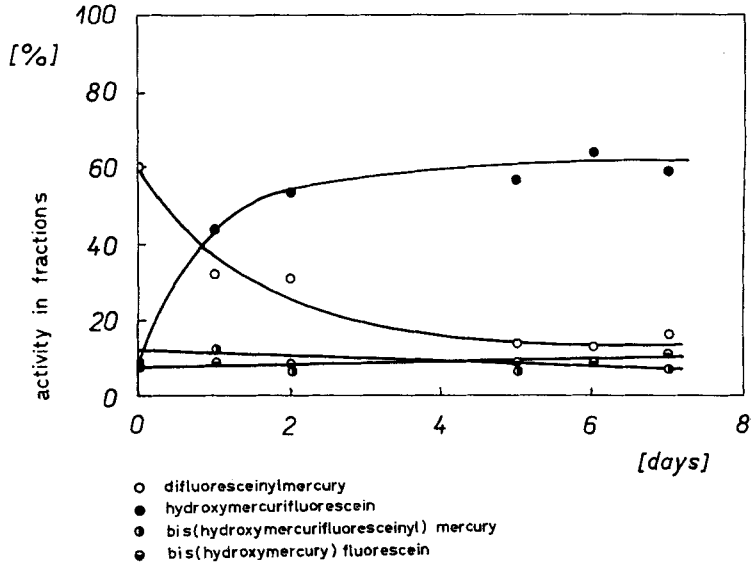

FIG. 5. The time dependence of the decomposition of difluoresceinylmercury-^{197}Hg.

mixture has been given the name «Mercurascan» and is used in further biological experiments. In the next work we attempted to work out a method for preparation of Mercurascan with higher yield.

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