

Ultra-microsynthesis of 5-Halogeno-derivatives of Uracil

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A method for preparation of 5-iodo-uracil has been worked out in ultra-micro-scale enabling the use of commercial ^{131}I at very high specific activity for the synthesis of 5- ^{131}I -uracil in microgram quantities.

The demonstration that 5-bromouracil could be introduced into the deoxyribonucleic acid (DNA) of some microorganisms, especially *Escherichia coli*, replacing up to 50 % of thymine units^{1,2}, has now been extended to comprise 5-chloro- and 5-iodouracil^{3,4}. With regard to relative rate of incorporation of the latter thymine analogues, Zamenhof *et al.*⁴ have found that 5-chlorouracil may replace 21 % of thymine units, 5-iodouracil 14 %, whereas no introduction of uracil itself could be demonstrated. These observations are of considerable interest in view of the possibility of introducing 5- ^{131}I -uracil into the DNA-structure in order to study the consequences of the subsequent nuclear decay $^{131}\text{I} \rightarrow ^{131}\text{Xe}$ *in situ*. This procedure might open up a new way of altering the chemical structure of DNA with the definite possibility of replacing some thymine-units by uracil, resulting from the decay of 5- ^{131}I -uracil. More detailed reports with regard to the general chemical aspect of this decay reaction will be published later.

In view of the fact that 1 Curie of ^{131}I corresponds to 7.7 μg of the pure element, the synthesis of substantial amounts of 5- ^{131}I -uracil with a minimum of diluting non-radioactive iodine has to be carried out in ultramicroscale. The following procedure has given up to 80 % yields of 5-iodouracil from 5 μg and less of uracil in the presence of an equivalent amount of iodine.

In principle the method is based on iodination of uracil in 0.02 M phosphate buffer at pH 7.5. Iodine is administered to the reaction mixture as a KI-solution, and liberated as iodine by the addition of an equivalent amount of chlorine in an aqueous solution. After completion of the reaction the products formed are separated by paper chromatography in a butanol-water system. In order to check the yield of 5-iodouracil and the amount and distribution of non-iodocompounds formed during the reaction, we have utilized uracil-6-¹⁴C of high specific activity as the starting material, integrating the activity distributed of spots along the chromatogram. By this way, amounts down to 0.02 μg could be determined with a fair degree of accuracy. Fig. 1 gives a picture of the distribution of reaction products from a run with uracil-6-¹⁴C and non-radioactive iodine as starting material, as compared with non-radioactive uracil and ¹³¹I as reaction partners. With regard to the latter combination ¹³¹I was

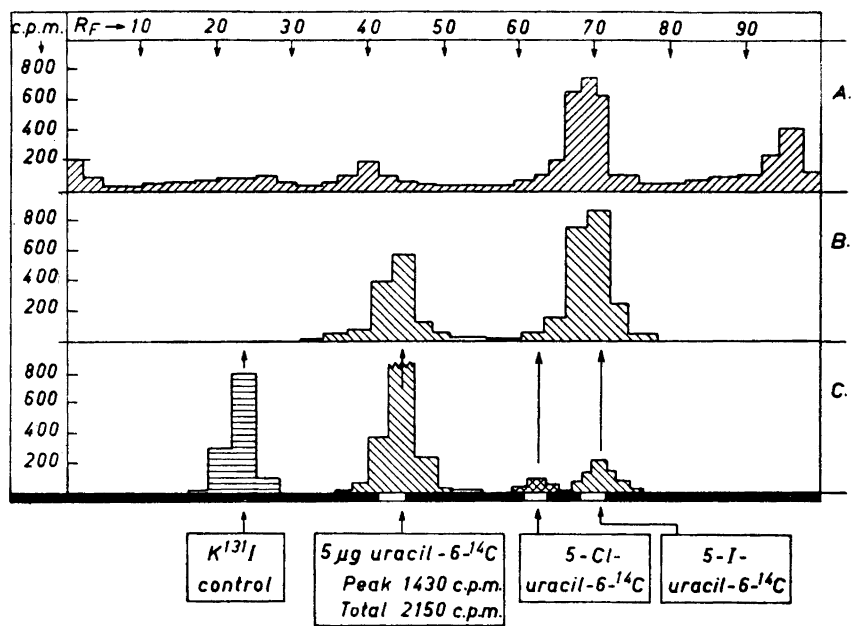


Fig. 1. Diagrammatic representation of the result of iodination of uracil in microgram quantities.

- A: Iodination of non-labelled uracil with ¹³¹I in tracer amounts followed by chromatographic separation of reaction products on a paper strip in butanol-water. Determination of radioactivity on sections of the paper strip gives a measure of the relative amounts of reaction products. Ordinate: counts per minute; abscissa: *R_F*-values.
- B: Analogue experiment with uracil-6-¹⁴C and non-radioactive iodine. The peak of 5-iodouracil corresponds to that of series A.
- C: Controls, showing the position of K¹³¹I, uracil-6-¹⁴C, 5-chlorouracil-6-¹⁴C and 5-iodouracil-6-¹⁴C. The position of controls in substantial amounts of the three last compounds are shown as white regions in the black base-line, the determination being based on photography in UV-light.

used in tracer amounts during the working out of optimum conditions for the synthesis. The preliminary runs were carried out with uracil-6-¹⁴C and non-radioactive iodine.

As seen from Fig. 1 the peak of activity corresponding to 5-I-uracil-6-¹⁴C coincides with that of 5-¹³¹I-uracil. Both are clearly separated from uracil, as was also verified by parallel controls of substantial amounts of uracil and 5-iodouracil (indicated by photography in UV-light at 254 m μ). With regard to the possible formation of small amounts of 5-chlorouracil a separate control with 5-chlorouracil-6-¹⁴C indicates a fair separation from 5-iodouracil-6-¹⁴C. In the experiments with ¹³¹I we observed, in addition to the peak corresponding to 5-iodouracil, four other peaks of lower activity. Two of them have been found to correspond to KI and iodine, one remains at the starting line of the chromatogram and one follows the locus of uracil. None of the peaks, however, interferes with the locus of 5-iodouracil of $R_F = 0.68 - 0.72$.

EXPERIMENTAL

Uracil-6-¹⁴C was prepared according to Fritzson⁵. It gave when chromatographed in 1 μ g amounts on paper in butanol-water a total count of 600 cpm per spot. It contained 2 % 5-bromouracil.

¹³¹I, as was used in these experiments, was derived from a tellurium source, together with some amounts of shortlived iodine isotopes, mainly ¹³²I. By the ordinary procedure of aeration of the tellurium solution the iodine was driven off and absorbed in a solution of 72.5 μ g KI in 2.5 ml of 0.02 M phosphate buffer, pH = 7.5.

As a standard procedure, the following reagents were introduced into a 2 ml test tube, which could be attached to a small condenser by a standard glass joint. First 500 μ l of a 0.02 M phosphate buffer solution, pH 7.5, containing 14.5 μ g KI was added to the reaction tube, followed by 5–10 μ l of a freshly made and standardized solution containing 3.5 μ g of chlorine and 5 μ g of uracil in 25 μ l of water. The condenser was then attached to the tube and the mixture was immediately heated to gentle boiling on a micro-heating stage for 5 min. After cooling to room temperature another addition of chlorine was made, corresponding to 1.7 μ g, followed by another 5 min period of heating. After cooling the reaction mixture was evaporated as a small spot on a paper strip, 23 mm wide, about 10 cm from one end. Whatman No. 1 paper was used, and the chromatogram run in a descending system of butanol saturated with water at 22°C for 18 h. Suitable controls of uracil-6-C, 5-I-uracil-6-¹⁴C, ¹³¹KI were made for the exact localization of the iodouracil from the reaction mixture.

After localization, by radioactivity measurement of controls, of the region containing the iodouracil formed, this region could be quantitatively eluted by 0.01 N HCl and almost quantitatively by water. Controls run in 20 μ g scale show the exact UV absorption curve of 5-iodouracil, with an E_{\max} at 283 m μ and E_{\min} at 246 m μ . Table 1 gives the absorption maxima and minima for uracil and some of its halogen-derivatives (solvent: 0.01 N HCl) obtained in this laboratory.

Compound	λ_{\max}	λ_{\min}
Cl-uracil	273	239
Br-uracil	273	240
I-uracil	283	246
Uracil	260	228

The preparation of 5-bromouracil could be carried out as a simple variation of the procedure outlined above; the yields were about 50 %.

With regard to the preparation of 5-¹³¹I-uracil using substantial amounts of ¹³¹I in high concentration, adequate shielding should be used; 5 cm of lead give sufficient protection. The above method is suitable for the direct use of commercial solutions of ¹³¹I in phosphate buffer for the reaction. Utilizing uracil-6-¹⁴C of the highest possible activity together with substantial amounts of ¹³¹I, the resulting 5-¹³¹I-uracil-6-¹⁴C could be used for the study of the nature of non-iodinized derivatives related to uracil, as formed in the course of the decay ¹³¹I → ¹³¹Xe.

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