MICRO-SCALE PREPARATION OF 4-THIOURACIL LABELLED WITH ^{14}C OR $^{14}C + ^{35}S$.

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SUMMARY

A selective and quantitative method was developed for a micro-scale preparation of 4-thiouracil-2-^{14}C by the direct thiation of uracil-2-^{14}C. From pyridine, dioxane, and tetralin as the thiation solvents, the best yield was obtained in dioxane (96.6% of 4-thiouracil-2-^{14}C). The simultaneous labelling with $^{14}C + ^{35}S$ was obtained by isotope exchange with elemental sulfur-^{35}S on refluxing in dimethylformamide. The present procedure may be used as a general method for the preparation of ^{14}C or $^{14}C + ^{35}S$ labelled bases of nucleic acids of both the pyrimidine and purine type as well as the corresponding nucleosides and nucleotides.

INTRODUCTION

The direct thiation with phosphorus pentasulfide was widely used in the preparation of unlabelled nucleoside and nucleotide thioderivatives[1-4] as well as the thioderivatives of the corresponding bases of both the pyrimidine and purine type[5,6].

A detailed analysis of the thiation method has been now effected in the present paper in connection with the pre-

paration of ^{14}C labelled thiouracil; this compound is the object of a growing interest in the biochemistry of nucleic acids[7,8]. Special attention was paid to the influence of working conditions on the course and yields of the thiation, because of unsatisfactory yields with the commonly used pyridine[9], dioxane[10] and tetralin[6,11] as reaction media. The additional labelling with ^{35}S was effected by isotope exchange with elemental sulfur-^{35}S in boiling dimethylformamide; this general method was developed in our Laboratory some time ago[12].

As it may be inferred from the earlier papers[1-6], the present experimental procedure can be used as a general method in the micro-scale preparation of ^{14}C-labelled bases of nucleic acids of both the pyrimidine and purine type, as well as their nucleosides and nucleotides, or in the simultaneous labelling of these substances with the ^{14}C and ^{35}S radionuclides.

EXPERIMENTAL

Uracil (Lachema, Czechoslovakia) was purified by repeated recrystallisations from water; phosphorus pentasulfide Pure (Lachema) was purified by extraction with carbon disulfide in a Soxhlet thimble and dried in vacuo; dioxane Analytical Grade (Lachema) was used directly; pyridine Analytical Grade (Hajduki, Poland) was dried over sodium hydroxide pellets and distilled; tetralin Pure (Lachema) was dried over sodium hydroxide pellets and distilled under diminished pressure; dimethylformamide Analytical Grade (Lachema) was dried over phosphorus pentoxide and distilled under diminished pressure;

uracil-2-^{14}C (55.4 mCi/mM) was obtained from the Institute for Research, Production and Application of Radioisotopes, Czechoslovakia; elemental sulfur-^{35}S (4.9 mCi/mg) was purchased from V/O Izotop, Soviet Union.

Descending paper chromatography was performed on Whatman No 3 paper in the solvent systems S_1, 1-butanol - acetic acid - water (10:1:3), and S_2, 2-propanol - concentrated aqueous ammonia - water (7:1:2). Two-dimensional ascending thin-layer chromatography was performed on ready-for-use indicator containing Silufol UV_{245} foils (Kavalier, Czechoslovakia) with the use of the solvent system S_1 for one direction and S_3, 1-butanol - 2.5% aqueous ammonia (86:14), for the other direction.

Column chromatography was carried out on Bio-Gel P2 100--200 mesh (Calbiochem, U.S.A.); column length, 760 mm; diameter, 17 mm; flow rate, 0.20-0.22 ml of 0.002 M triethylammonium hydrogen carbonate per min. The course of the column chromatography was checked by a simultaneous continuous radioactivity and ultraviolet absorption measurement on a device according to Tykva and Grünberger[13].

In model experiments, the reaction mixture containing 1.0 mg (8.9 micromol) of uracil-2-^{14}C (0.2 mCi/mM), phosphorus pentasulfide, and 0.3 ml of the solvent was refluxed in a micro test tube equipped with a reflux condenser and protected from the atmospheric moisture by a drying tube. The molar ratio of uracil to phosphorus pentasulfide was in the range from 2:1 to 1:4. Samples (volume, 10 µl) of the reaction mixture were withdrawn in time intervals depending on the thiation rats in the particular solvent (Fig. 1), subjected to paper

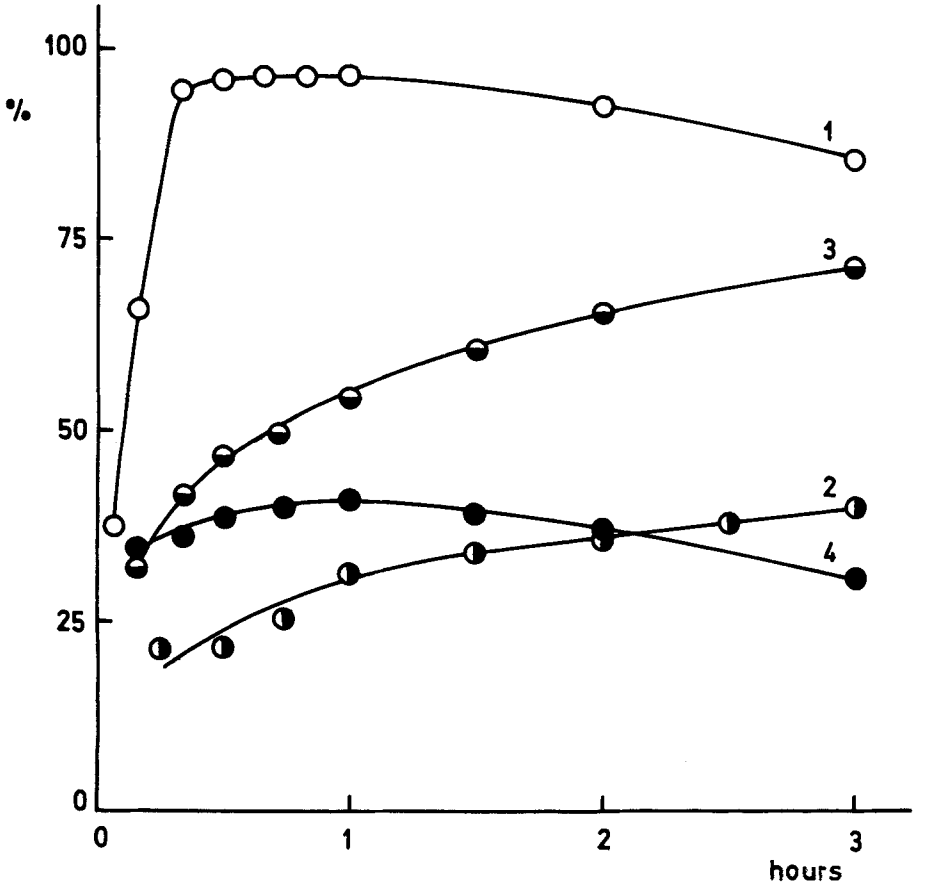

FIG. 1

Time Dependence of Yields of 4-Thiouracil-2-^{14}C.

Solvent (0.3 ml) : ○ dioxane; ◑ pyridine; ◒ pyridine; ● tetralin. Molar radio uracil - phosphorus pentasulfide: ○ 1:2; ◑ 1:2; ◒ 1:4; ● 2:1.

chromatography in the solvent system S_1, and radiometrically evaluated.

The radioactivity distribution on paper chromatograms was determined on an automatic device having two GM counters with thin end windows in the 4π geometry (Frieseke Hoepfner, German Federal Republic). Molar specific activities were determined by means of a spectrophotometer (Model SF - 4, Soviet Union) and Tri-Carb liquid scintillation spectrometer (Model 3375; Packard, U.S.A.).

The radiometric detection of the substance labelled simultaneously with ^{14}C and ^{35}S was effected by semiconductographic processes[14] using silicon semiconductor detectors developed in this Laboratory[15] and making possible a simultaneous non-destructive determination of both radionuclides in a two-channel device[16,17] ("carbon" channel, 9-156 keV; "sulfur" channel, 160-170 keV).

RESULTS AND DISCUSSION

The results of model experiments (Fig. 1) are expressed as a plot of the 4-thiouracil-2-^{14}C yield on the time of the reaction in the particular solvent. The best yield of the reaction was obtained in dioxane (Fig. 1, curve 1). As early as after 50 minutes refluxing, the reaction mixture contains the maximum yield (96.6%) of the product thiated at position 4, while the proportion of the subsequent thiation at position 2 is almost negligible (1.7% of 2,4-dithiouracil-2-^{14}C). The time dependence of the composition of the reaction mixture is shown in Table I.

The reaction course in pyridine as solvent was examined

TABLE I

Time Dependence of the Composition of Reaction Mixtures in Dioxane as Solvent

Reaction time (min)	U (%)	4-TU (%)	2,4-DTU (%)
10	38.1	61.8	0.1
20	4.1	94.8	1.1
30	2.5	96.2	1.3
40	2.2	96.4	1.4
50	1.9	96.6	1.5
60	1.7	96.6	1.7

Dioxane, 0.3 ml. The ratio uracil - phosphorus pentasulfide, 1:2. U, uracil-2-^{14}C. 4-TU, 4-thiouracil-2-^{14}C. 2,4-DTU, 2,4-dithiouracil-2-^{14}C.

with the use of two different ratios of reactants (Fig. 1, curves 2 and 3). Also in this case, the extent of the additional thiation at position 2 is low (only 4.7% of the dithio derivative is formed with the molar ratio 1:4 of uracil to phosphorus pentasulfide). On the other hand, the yields of 4-thiouracil are in pyridine considerably lower than in dioxane even with the use of a fourfold molar excess of phosphorus pentasulfide (74.2% of 4-thiouracil). Moreover, the preparative application of the pyridine procedure on a microscale is

considerably complicated by the isolation of the isoluble tarry thio derivative from the reaction mixture.

As it is known from earlier papers[11], the thiation in tetralin is not selective, being accompanied by the additional thiation at position 2. Thus with the ration 2:1 of uracil to phosphorus pentasulfide, the maximum yield of 4-thiouracil (40.9%) is obtained when the reaction time is one hour (5.3% of the dithio derivative is formed in the side reaction). Additional refluxing results in decreasing yields of 4-thiouracil in favour of 2,4-dithiouracil (Fig. 1, curve 4). A higher proportion of phosphorus pentasulfide in the reaction mixture leads to a quantitative formation of 2,4-dithiouracil (after 30 min of refluxing with the ratio 1:1 of uracil to the pentasulfide).

In the preparation of 4-thiouracil-2-^{14}C, there was used 0.50 mg (4.45 micromol) of uracil-2-^{14}C (55.4 mCi/mM; radiochemical purity 99%, as determined by thin-layer chromatography), 2.0 mg (8.9 micromol) of phosphorus pentasulfide, and 0.3 ml of dioxane. The reaction mixture was refluxed for one hour, transferred into a flask and coevaporated to dryness with 5 ml of 0.1% aqueous ammonia. The residue was dissolved in water (1 ml) and the solution subjected to column chromatography (Fig. 2). The 4-thiouracil-2-^{14}C-containing fractions were combined and evaporated to dryness. The residue was coevaporated with three 5 ml portions of methanol to remove traces of triethylammonium hydrogen carbonate. The radiochemical purity was 99.6%, as determined by two-dimensional thin-layer chromatography. Yield, 0.37 mg (3.30 micromol; 74%) of 4-thiouracil-2-^{14}C (55.4 mCi/mM); ultraviolet absorption maximum

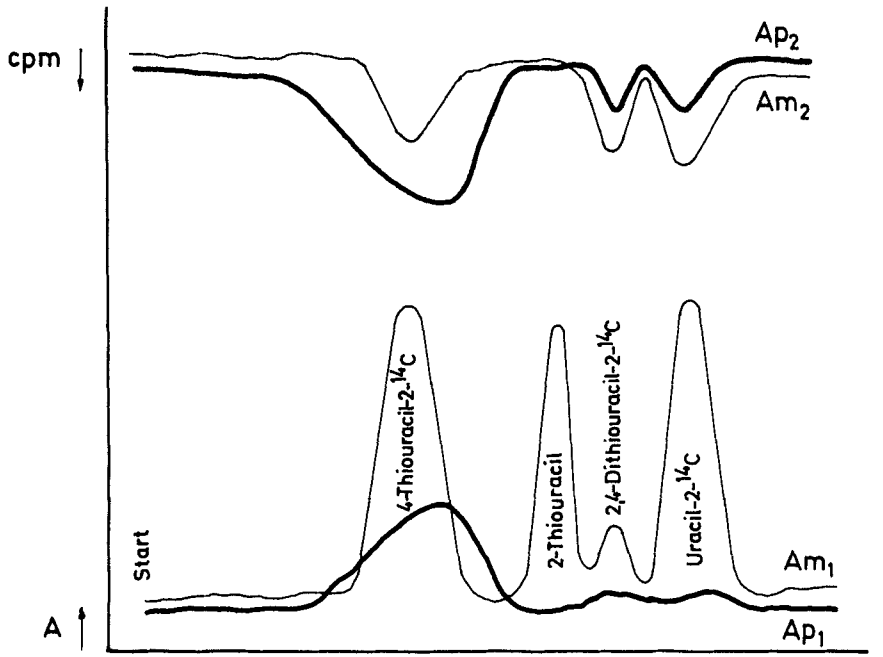

FIG. 2

Record of the Separation of a Mixture of Uracil and its Thioderivatives on a Chromatographic Column.

Model mixture: Am_1 absorbance; Am_2 radioactivity; actual reaction mixture: Ap_1 absorbance; Ap_2 radioactivity.

(pH 7): 330 nm. The identity of this substance was confirmed by comparison with inactive 4-thiouracil on paper chromatography in the solvent systems S_1, S_2, and S_3.

The thus-obtained 4-thiouracil-2-^{14}C was used for the preparation of 4-thiouracil, simultaneously labelled with

the radionuclides ^{14}C and ^{35}S. Thus, a mixture of 4-thiouracil-2-^{14}C (100 /uCi; 55.4 mCi/mM), dimethylformamide (1.0 ml), and elemental sulfur-^{35}S (1.5 mCi; 2.6 mCi/g) was heated at 155 °C for two hours, allowed to cool, and paper-chromatographed (descending technique) in the solvent system S_2. Elution afforded 4-thio-^{35}S-uracil-2-^{14}C, which was then purified by column chromatography. The thioderivative-containing fractions were evaporated and the residue taken down with three 5 ml portions of methanol on a rotatory evaporator to remove traces of triethylammonium hydrogen carbonate to afford 1.2 /umol of 4-thio-^{35}S-uracil-2-^{14}C, (55.4 mCi ^{14}C/mM; 52.0 mCi ^{35}S/mM) in 66.8% yield; the radiochemical yield of the exchange was 69%. The identity of the substance was confirmed by comparison with an authentic specimen of inactive 4-thiouracil on paper chromatography in the solvent system S_1, S_2, and S_3.

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