Synthesis of 5-Trifluoromethyluracil-2-^{14}C*

Arthur J. ZAMBITO and Earl M. CHAMBERLIN

Merck Sharp and Dohme Research Laboratories
Division of Merck and Co., Inc.
Rahway, New Jersey, U. S. A.
Received April 19, 1971

SUMMARY

Diethyl ethoxymethylenemalonate was condensed with thiourea-^{14}C in the presence of sodium ethylate to give ethyl 2-thio-6-oxypyrimidine-5-carboxylate-2-^{14}C. Desulfurization of the latter pyrimidine with chloroacetic acid gave uracil-5-carboxylic acid-2-^{14}C, which was subsequently fluorinated with sulfur tetrafluoride and hydrogen fluoride to afford the desired 5-trifluoromethyluracil-2-^{14}C in 25.6% overall yield based on thiourea.

INTRODUCTION.

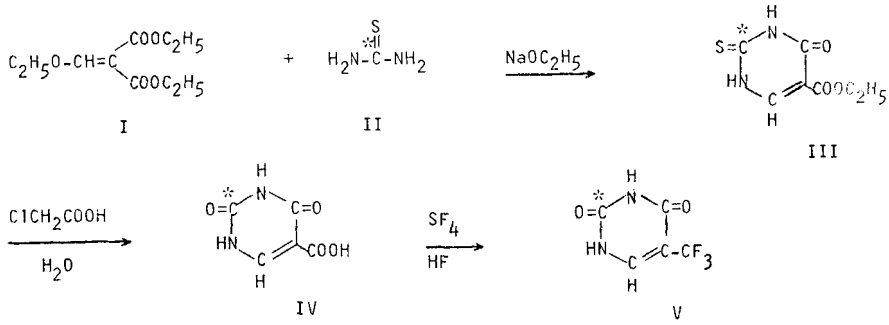
Heidelberger *et al.* [1] first synthesized 5-trifluoromethyluracil and the corresponding radioactive 5-trifluoromethyluracil-2-^{14}C as a logical sequence to his earlier synthesis of 5-fluorouracil [2, 3] which was found to possess certain interesting biological properties. As an antimetabolite this compound exhibited antitumor activity against mouse and human tumors [4, 5]; it was also convertible into 5-fluoro-2′-deoxyuridine-5′-monophosphate, an inhibitor of the enzyme thymidylate synthetase [6, 7]; and it was shown to be involved in other biochemical changes of interest, such as incorporation into RNA [8, 9, 10, 11].

The radioactive compound was prepared via a multi-step syntesis in 5.9% overall yield, starting with barium carbonate-^{14}C which was converted to urea-^{14}C, subsequently used as the acetyl derivative in condensation with the key intermediate, β-bromo-α-trifluoromethylpropionamide. The resulting labeled acetylated ureidoamide was cyclized to 5-trifluoromethyl-5,6-dihydro-

* This investigation was supported by Contract PH43-62-479 with Chemotherapy, National Cancer Institute, National Institutes of Health.

uracil-2-^{14}C, which after bromination and dehydrobromination gave 5-trifluoromethyluracil-2-^{14}C.

In the reaction sequence outlined below we present a more direct route to the radioactive compound (V). The method of Ballard and Johnson [12] (I → IV) was used to prepare uracil-5-carboxylic acid-2-^{14}C (IV), which in turn was converted to the desired V by a modification of the procedure of Mertes and Saheb [13].



The result is a convenient three step synthesis of 5-trifluoromethyluracil-2-^{14}C (V). Four hundred and sixty milligrams were prepared in 25.6% yeild. The specific activity was 40.0 microcuries per milligram, starting with 760 mg of thiourea which contained 550 mg of thiourea-^{14}C, having an activity of about 60 millicuries.

Experimental.

Melting points were not corrected. The radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer, Model 3315 (Packard Instruments Co., Inc., Downers Grove, Ill., U. S. A.). The liquid scintillation phosphor solution consisted of 4 g of 2,5-diphenyloxazole (PPO) and 0.3 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP) per liter of toluene-ethanol, 70-30. One ml of methanol solution or 0.5 ml of aqueous solution of radioactive compound was added to 19.5 ml of phosphor solution in glass counting vials.

Paper chromatography was carried out by the descending technique on Whatman No. 1 paper in the system *n*-butanol/acetic acid/water (50/20/30). Visualized over UV light (short λ), the chromatogram showed a main UV absorbing area and a faint trailing impurity, containing about 0.5% of the total radioactivity as measured by the sectioning and direct counting procedure described below. Before cutting the strip, it was scanned on a Packard Radiochromatogram Scanner Model 7201. No radioactivity other than these two

areas was detectable. On the same paper strip the quantitization of the radioactivity was determined by cutting the strip into 3 cm sections which were placed in liquid scintillation counting vials to which was added phosphor solution and counted directly. Using a separate paper strip, the main UV absorbing area was cut into three sections and each eluted with 0.1 *N* HCl. The specific activity of each section was determined by UV absorption and counting an aliquot of the eluate. These specific activities were found to be constant and equal to that of the bulk sample. (The UV measurements were performed on a Cary UV spectrophotometer, Model 11.)

Ethyl 2-thio-6-oxypyrimidine-5-carboxylate-2-^{14}C (III).

Thiourea-^{14}C (II), 550 mg. (about 60 mC) (Merck Sharp & Dohme Canada Limited, Montreal, Canada) was diluted with 210 mg of nonradioactive reagent thiourea (Matheson Coleman and Bell, East Rutherford, N. J., U. S. A.) to give 760 mg. (0.01 mole). This was added to a magnetically stirred solution of 250 mg. (0.01085 mole) of sodium in 40 ml. of absolute ethanol, and to the resulting solution was added slowly dropwise 2.25 g. (2.1 ml., 0.0104 mole) of freshly distilled diethyl ethoxymethylenemalonate (I, $n^{23} = 1.4595$, Kay-Fries Chemicals, Inc.. New York City, U. S. A.). The reaction was exothermic, and the solution turned yellow with precipitation of solid. After the addition was completed, the mixture was refluxed one hour, allowed to cool to room temperature and filtered. The light yellow solid was dried at 65°/0.1mm./P_2O_5/ one hour to give 2.25 g. This crude sodium salt was dissolved in 100 ml. of water at 40° C, cooled to 30° C and neutralized to pH 4 with dilute hydrochloric acid. The impure thioester III, after filtering, washing with a minimum of cold water, and drying as previously, weighed 1.67 g. It was recrystallized from approximately 100 ml. of boiling water (50 mg. Darco KB, Atlas Chem. Co., New York City, U. S. A.) to give 1.53 g. of ethyl 2-thio-6-oxypyrimidine-5-carboxylate-2-^{14}C (III); m.p. 253-255° d (sealed tube); lit. [12] m.p. 245° C.

Uracil-5-carboxylic Acid-2-^{14}C. (IV)

The above 1.53 g. of III was refluxed with stirring in a solution of 1.53 g. of chloroacetic acid in 12.5 ml. of water for three hours, after which the solution was evaporated to dryness on the steam bath and then co-evaporated with water three times. The white residue was recrystallized from about 65 ml. of bloiling water (50 mg. Darco KB). The filtrate was reheated to dissolve rapidly precipitated product, cooled to room temperature, and then stored at 5° C overnight. After drying the filtered product in the usual manner, it weighed 1.055 g. Two additional recrystallizations from approximately 25 ml. of water gave 810 mg. of purified uracil-5-carboxylic acid-2-^{14}C (IV); m.p. 278-280° C (sealed tube); lit. [12] m.p. 268-270° C.

5-Trifluoromethyluracil-2-^{14}C (V).

A mixture of 810 mg. of uracil-5-carboxylic acid-2-^{14}C (IV), 3.0 g. of sulfur tetrafluoride (Du Pont Chemical Co., Wilmington, Del., U. S. A.) and 2 ml. of hydrogen fluoride (Matheson Gas Products, East Rutherford, N. J., U. S. A.) was heated in a 10 ml. stainless steel bomb with agitation for three hours at 100° C. The bomb was cooled to room temperature, cautiously vented in a hood, and after vigorous escape of gaseous products had subsided, the green reaction mixture was poured into a small polyethylene dish. The bomb was rinsed thoroughly with liquid hydrogen fluoride, and the mixture allowed to evaporate to dryness. A solution of the light colored residue in 10 ml. of boiling water was decolorized (50 mg. of Darco KB), filtered and the charcoal cake washed with a few milliliters of hot water. The combined filtrate and washings was reheated to effect solution and then allowed to cool slowly to room temperature. After cooling for one hour in an ice bath, the product was filtered off, transferred completely from the flask with the filtrate, and finally washed with a minimum of cold water. The yield of white crystalline product, dried at 65°/0.1 mm./P_2O_5, was 570 mg. After a second recrystallization from 6 ml. of hot water (without charcoal), 460 mg. of 5-trifluoromethyluracil-2-^{14}C (V) was obtained; 25.6% overall yield based on thiourea; m.p. 252-254° C, d. (sealed tube); lit. [13] m.p. 247-249° C, d. The specific activity was 40.0 μC/mg. A paper strip chromatogram was essentially single spot (*n*-butanol/acetic acid/water - 50/20/30). Ultraviolet : λ max in mμ ($\varepsilon = 10^{-3}$); in 0.1 *N* HCl, 256.5 (8.1); in pH 7, 258 (7.35); in pH 8.1, 279 (7.13); lit. [13] 257 (8.15); 257 (7.21); 279 (6.5).

ACKNOWLEDGEMENT.

We gratefully acknowledge Mr. Henry T. Meriwether for performing the activity measurements, the ultraviolet spectra, and the paper chromatography.

REFERENCES

1. HEIDELBERGER, C., PARSONS, D. G. and REMY, C. D. — *J. Med. Chem.*, **7** : 1 (1964).
2. HEIDELBERGER, C., CHAUDHURI, N. K., DANNEBERG, P., MOOREN, D., GRIESBACH, L., DUSCHINSKY, R., SCHNITZER, R. J., PLEVEN, E and SCHEINER, J. — *Nature*, **179** : 663 (1957).
3. DUSCHINSKY, R., PLEVEN, E. and HEIDELBERG, C. — *J. Am. Chem. Soc.*, **79** : 2559 (1957).
4. HEIDELBERGER, C., GRIESBACK, L., MONTAG B.J., MOOREN, D., CRUZ O., SCHNITZER, R.J. and GRUNBERG, E. — *Cancer Res.*, **18** : 305 (1958).
5. ANSFIELD, F. J., SCHROEDER, J. M. and CURRERI, A. R. — *J. Am. Med. Assoc.*, **181** : 295 (1962).
6. HEIDELBERGER, C., KALDOR, G., MUKHERJEE, K. L. and DANNEBERG, P.B. — *Cancer Res.*, **20** : 903 (1960).

7. HARTMAN, K.-U. and HEIDELBERGER, C. — *J. Biol. Chem.*, **236** : 3006 (1961).
8. CHAUDHURI, N. K., MONTAG, B. J. and HEIDELBERGER, C. — *Cancer Res.*, **18** : 318 (1958).
9. CHAUDHURI, N. K., MUKHERJEE, K. L. and HEIDELBERGER, C. — *Biochem. Pharmacol.*, **1** : 328 (1958).
10. MUKHERJEE, K. L. and HEIDELBERGER, C. — *J. Biol. Chem.*, **235** : 433 (1960).
11. MUKHERJEE, K. L., CURRERI, A. R., JAVID, M. and HEIDELBERGER, C. — *Cancer Res.*, **23** : 67 (1963).
12. BALLARD, E. and JOHNSON, T. B. — *J. Am. Chem. Soc.*, **64** : 794 (1942).
13. MERTES, M. P. and SAHEB, S. E. — *J. Pharm. Sci.*, **52** : 508 (1963).