AN IMPROVED SYNTHESIS OF 5-FLUOROURACIL-6-^{14}C

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SUMMARY

Uracil-6-^{14}C (7), prepared in high radiochemical yield by conventional methods, has been converted to 5-fluorouracil-6-^{14}C (9) in over 90% yield using trifluoromethylhypofluorite. The use of this recently introduced reagent eliminates the necessity of preparing 9 from fluorinated precursors.

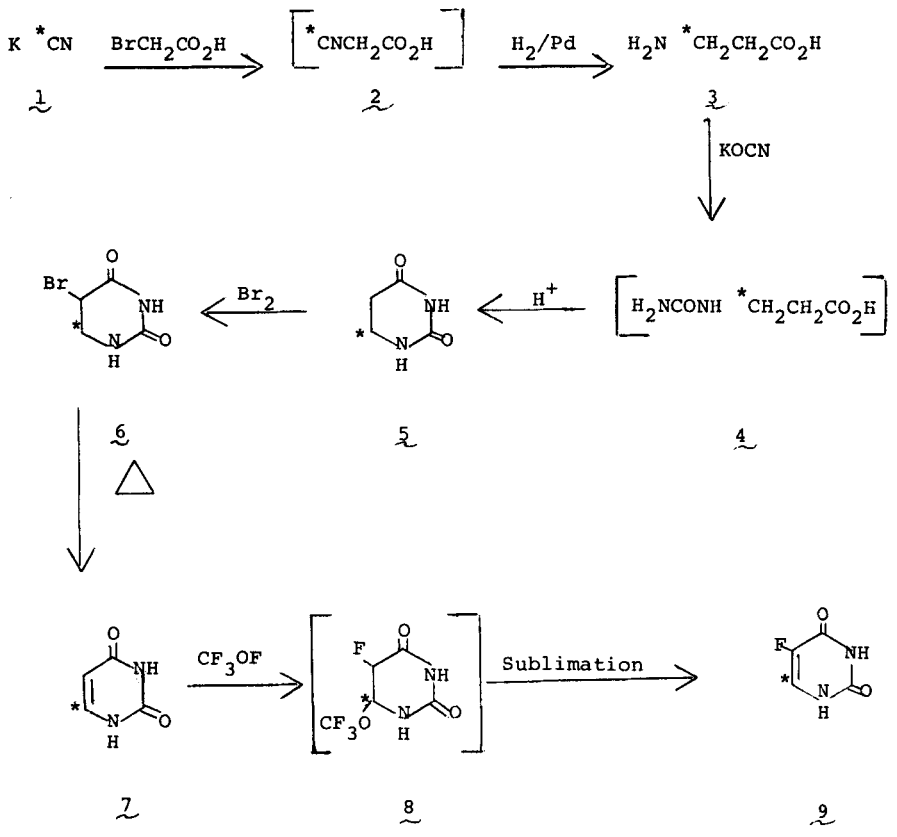
Introduction and Discussion

A number of radioisotopic syntheses of 5-fluorouracil (9) have been published since the introduction of this compound as a medicinal agent. The original synthesis[1] constructs the fluorinated pyrimidine ring from the condensation product of ethyl formate and ethyl fluoroacetate and a urea derivative, thereby enabling a carbon-14 label to be inserted in one or more of the four carbon atoms of the final compound. In actual practice, 5-fluorouracil-2-^{14}C,[2] derived from O-methylisourea-^{14}C or a thiourea-^{14}C,[3] and 5-fluorouracil-6-^{14}C[4] obtained *via* ethyl formate- have been more readily prepared by this synthesis. In addition, specifically labelled 5-fluorouracil-6-^{3}H[5] and 5-fluorouracil-^{18}F[6] have been obtained from preformed pyrimidine precursors.

For biological evaluation of 5-fluorouracil, a carbon-14 label at C_6 is more desirable than at C_2 since the former is considerably more stable metabolically. Both derivatives are

prepared by the same synthetic sequence, but differ greatly in radiochemical yield. Thus, 5-fluorouracil-2-^{14}C can be prepared from barium carbonate-^{14}C in 5 steps in overall radiochemical yield of about 60% while it requires six steps to provide the $^{14}C_6$ derivative in radiochemical yield of 10% from barium-^{14}C-carbonate. This lower yield is a reflection of an early labelled intermediate, ethyl formate-^{14}C, required as an excess reagent in the synthesis. Conceivably the biologically stable $^{14}C_5$ derivative could be prepared in higher radiochemical yield but it would require ethyl fluoroacetate-2-^{14}C, which is difficult to prepare, as the ^{14}C carrier.

SCHEME



While the existing method of preparing 5-fluorouracil-6-^{14}C does provide the compound unambiguously, the relatively low radiochemical yield prompted our investigation of an alternate synthesis which is now reported. (Scheme)

Since uracil (7) has recently been reported[7] to undergo high yield fluorination with trifluoromethyl hypofluorite to form 5-fluorouracil, the necessity of carrying out a labelled synthesis from fluorinated precursors no longer exists. Uracil, labelled in a variety of positions, can be prepared in excellent yield by well established procedures. We chose the C_6 labelling since it would provide material for metabolic study consistent with previously prepared 5-fluorouracil-6-^{14}C.

5,6-Dihydrouracil (5) is readily obtained by acid catalyzed cyclization of 3-ureidopropionic acid (4) which is prepared[8] from potassium cyanate and β-alanine. The latter is readily obtained by hydrogenation of cyanoacetic acid,[9] prepared from potassium cyanide and bromoacetic acid. Thus the dihydropyrimidine (5) is obtained in four steps in overall yield of approximately 80% and with the capability of specifically labelling the 4,5, or 6 positions. Since an excess of potassium cyanate is used, this approach to $^{14}C_2$-5-fluorouracil offers no advantage over the existing method.[10]

Attempts to fully aromatize 5,6-dihydrouracil with Pd/C were unsuccessful. A good yield of uracil could be obtained by treatment of 5 with alloxan;[11] however, the resulting products are not readily separable. The long standing method of carrying out this conversion by bromination—dehydrobromination[8] of 5, while not totally unambiguous, was used and provided uracil-6-^{14}C in 85% yield after crystallization. Fluorination to 5-fluorouracil-6-^{14}C was carried out in a modification of the existing procedure[7] in over 90% yield and an overall radiochemical yield of 60%. While this synthesis requires several more steps, several of the

intermediates do not have to be isolated and the overall yield compares quite favorably with labelled 5-fluorouracil obtained by other methods.

Experimental

Melting points are uncorrected. All solvents used were distilled. Radioactivity was measured by the liquid scintillation technique using a Packard Tricarb Model 2010 spectrometer. Radiochemical purity where indicated was determined by thin layer chromatography using EM precoated silica gel 60 F254 tlc plates developed with ethyl acetate-acetone (9:1). The plates were scanned on a Packard Model 7201 Radiochromatogram Scanner System.

Potassium cyanide-^{14}C (1)- Barium carbonate-^{14}C (1 mmole, 60 mCi) was treated with potassium azide and potassium metal under fusion conditions essentially as described[12] to provide K^{14}CN in 80-90% yield.

Cyano-^{14}C-acetic acid (2)- The potassium cyanide-^{14}C obtained above from 2 mmoles of Ba$^{14}CO_3$ was treated with 0.1N silver nitrate solution (16 ml total) to precipitate silver cyanide-^{14}C which was filtered then washed with 0.02N HNO_3 followed by distilled water. The moist precipitate was then stirred for 2 hours at 50° with 2.5 ml of 1N HCl and the liberated H^{14}CN was flushed with H_2 into a collecting trap cooled to -190°C. The H^{14}CN was then transferred under high vacuum into a solution of 306 mg (2.2 mmole) of bromoacetic acid and 162 mg (4.05 mmole) of sodium hydroxide in 2 ml of water and the resulting mixture was stirred at 60° for 45 minutes. After cooling, any unreacted H^{14}CN was removed by vacuum transfer. The reaction was found to yield over 90% of product which required no further purification.

3-Aminopropionic-3-^{14}C acid (3)- The solution of crude cyanoacetic acid was added over a 1 hour period to a mixture of 10 ml of 1N HCl and 100 mg of 10% Pd/C in an atmosphere of hydrogen. Uptake of hydrogen was complete 1.5 hour after all of the cyanoacetic acid had been added. After this time, the mixture was filtered and the filtrate concentrated to dryness *in vacuo*. The residue was dissolved in water and the product purified by ion exchange chromatography in essentially the same manner as described.[13] A yield of 119 mg (1.33 mmole, 81%) was obtained.

5,6-Dihydrouracil-6-^{14}C (5)[8]- A solution of 119 mg of 3-aminopropionic-3-^{14}C acid and 170 mg (1.28 mmole) of potassium cyanate in 3 ml of water was slowly evaporated to dryness in an oil bath. The viscous residue was acidified with 1N HCl and again evaporated to dryness and then heated to 170°. Heating was continued for 0.5 hour. The mixture was cooled and 5,6-dihydrouracil-6-^{14}C was isolated by crystallization from water yielding 106 mg of product of >98% radiochemical purity. The mother liquor was purified by ion exchange chromatography and yielded a second crop of 36 mg for a total yield of 142 mg (1.24 mmole, 93.5%).

5,6-Dihydro-5-bromouracil-6-^{14}C (6)[8]- 5,6-Dihydrouracil-6-^{14}C (142 mg) was suspended in 2 ml of acetic acid. With stirring the mixture was heated to 105°. At this temperature and with continued stirring, a solution of 200 mg of bromine (1.25 mmole) in in 1.5 ml of acetic acid was added dropwise over a 30 minute period. Heating was continued until the reaction mixture became colorless. After cooling, the mixture was concentraded *in vacuo* and the residue crystallized from water to yield 204 mg (1.06 mmole, 85.4%) of 6 which was >98% radiochemically pure.

Uracil-6-^{14}C (7) - Dehydrobromination of 6 was effected quantitatively by heating the compound to 200°C for 30 minutes. The residue (117 mg, 1.05 mmole) was >98% radiochemically pure 7.

5-Fluorouracil-6-^{14}C(9)- A mixture of 117 mg of uracil-6-^{14}C, 4 ml of trifluoroacetic acid, 8 ml of water and 20 ml of trichlorofluoromethane was cooled to -30°C. With magnetic stirring, the mixture was flushed with nitrogen then trifluoromethylhypofluorite[7] was bubbled through the solution at a moderate rate for 5 minutes. The reaction mixture was then brought to room temperature over a 3 hour period and stirred for an additional hour. The mixture was then concentrated in vacuo and the residue sublimed under high vacuum to yield 131 mg (1.01 mmole) of product which was >98% radiochemically pure. Nonradioactive 5-fluorouracil was added to this material to give a specific activity of 30 mCi/mmole. The overall radiochemical yield from K^{14}CN was 63%.

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