NOTES

SYNTHESIS OF [Carboxyl - ^{14}C] 5 - FLUOROOROTIC ACID

Summary

[Carboxyl - ^{14}C] 5 - fluoroorotic acid was synthesized by the treatment of [carboxyl - ^{14}C] orotic acid with trifluoromethyl hypofluorite followed by triethyl amine.

Key Words: synthesis, [carboxyl - ^{14}C] 5 - fluoroorotic acid, trifluoromethyl hypofluorite

5 - Fluoroorotic acid is an effective agent for the inhibition of a wide variety of tumors in animals (1). After synthesizing [2-^{14}C] 5 - fluoroorotic acid, Chaudhuri, et al. (2) showed that this compound was incorporated into RNA but not DNA. For our enzymatic studies with 5 - fluoroorotic acid, however, we needed the ^{14}C-label in the carboxyl group of this compound.

The synthesis of 5 - fluoroorotic acid by the fluorination of orotic acid with trifluoromethyl hypofluorite followed by sublimation was reported previously (3), but in our hands, mixtures of 5 - fluoroorotic acid and 5 - fluorouracil were obtained. A modification of this procedure for the synthesis of fluorinated uracils (4), uracil nucleosides (4), and nucleotides (5) was reported by Robins et al. We found this procedure converted orotic acid in high yield to fluoroorotic acid without any contamination by fluorouracil.

We report here a simple synthesis of [carboxyl - ^{14}C] 5 - fluoroorotic acid starting from [carboxyl - ^{14}C] orotic acid.

Experimental Section

Melting points were taken on a Hoover-Thomas Uni-melt apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 283 spectrophotometer.

0362-4803/79/0216-0361$01.00

Received June 5, 1978
Revised July 17, 1978

Radioactivity was measured with a Packard Tri-Carb Model 3380 liquid scintillation counter. Chromatography was done on Whatman 3MM paper. Orotic acid monohydrate was purchased from Sigma Chemical Company, [carboxyl-^{14}C] orotic acid from New England Nuclear (50μCi; 0.25mCi/mg), 5-fluoroorotic acid from P-L Biochemicals, trifluoromethyl hypofluorite from PCR Research Chemicals, and Freon 11 from Matheson. Caution: trifluoromethyl hypofluorite is toxic and a strong oxidant and has been known to cause explosions (4).

[Carboxyl-^{14}C] 5-Fluoroorotic Acid

The following procedure is an adaptation of the procedure of Robins, et al. (4).

Orotic acid monohydrate (156 mg, 0.9 mmole) and [carboxyl-^{14}C] orotic acid monohydrate (0.2 mg, 50 μ Ci) was dissolved in methanol (250 ml) then cooled under a drying tube in a Dry ice-acetone bath. To this stirred solution was added a solution of trifluoromethyl hypofluorite (1g) in Freon 11 (20 ml) at -78° (obtained by slowly bubbling CF_3OF into Freon 11 in a Dry ice-acetone bath and periodically weighing the gas cylinder). The reaction was complete when the UV absorbance at 262 nm disappeared (<15 min.). Nitrogen was bubbled through the solution which was stirred at room temperature for 45 min., then the solvent was evaporated, giving a white solid. This solid was dissolved in Et_3N-MeOH-H_2O (10:45:45; 15 ml) and stirred at room temperature in the dark for 16 hr. The solvent of the gel-like mixture was evaporated and the residue transferred in 3ml H_2O with filtration to a centrifuge tube. HCl (6N) was added dropwise until no more solid precipitated. The solid was collected and washed with 2 x 1 ml of cold H_2O to yield a white powder (140 mg, 81%). The product was recrystallized from H_2O. When the reaction was performed in the absence of [carboxyl-^{14}C] orotic acid, the white solid gave a mp 253-256° (lit(6) 245-250°; lit(7) 255°; lit(3) 258-259°) and had an IR and UV spectrum identical with

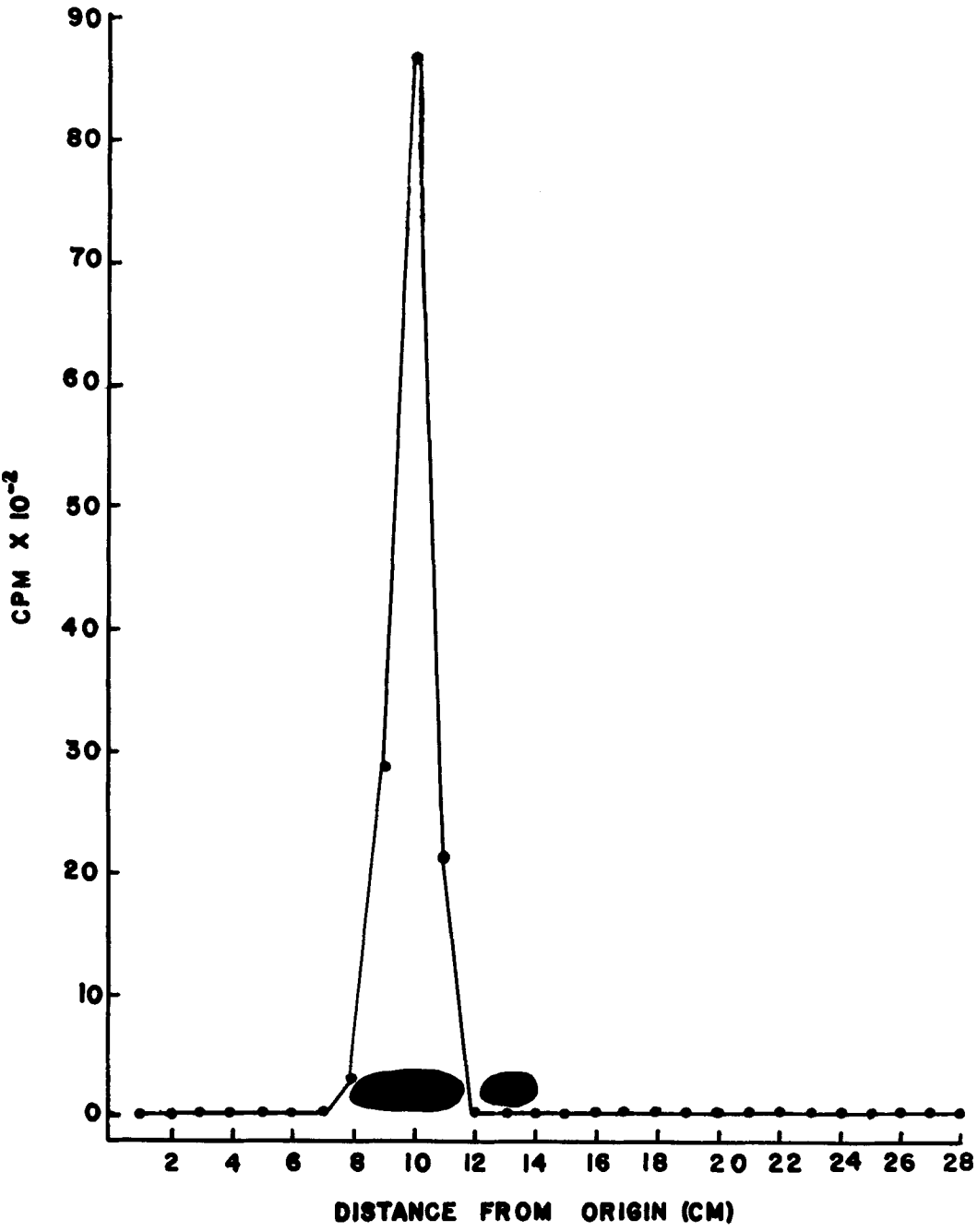

Figure 1. Radiopurity of the [Carboxyl - ^{14}C] 5 - Fluoroorotic Acid.

Separation was obtained by descending paper chromatography eluting with isopropanol - conc. NH_3-H_2O (7:1:2) (8). The spot at 10 cm is 5 - fluoroorotic acid and the one at 13 cm is orotic acid.

commercial 5-fluoroorotic acid. Descending paper chromatography eluting with isopropanol-conc. NH_3-H_2O (7:1:2) (8) gave one spot and all of the radioactivity corresponded to 5-fluoroorotic acid (see figure 1). The specific activity of the [carboxyl-^{14}C] 5-fluoroorotic acid was 2.1×10^5 dpm/μmole.

Acknowledgments

The authors would like to thank Dr. Malcolm MacCoss for helpful discussions about the fluorination procedure. This research was supported by a grant from the National Cancer Institute, DHEW(CA 21156) and from the Research Corporation.

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