SYNTHESIS OF CARBON-14 LABELED 1-β-D-ARABINOFURANOSYLCYTOSINE.

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SUMMARY

The synthesis of carbon-14 labeled 1-β-D-arabinofuranosylcytosine and its hydrochloride salt is described. The products, prepared in four steps from barium carbonate-^{14}C, are labeled in the C-2 position of the pyrimidine ring.

INTRODUCTION

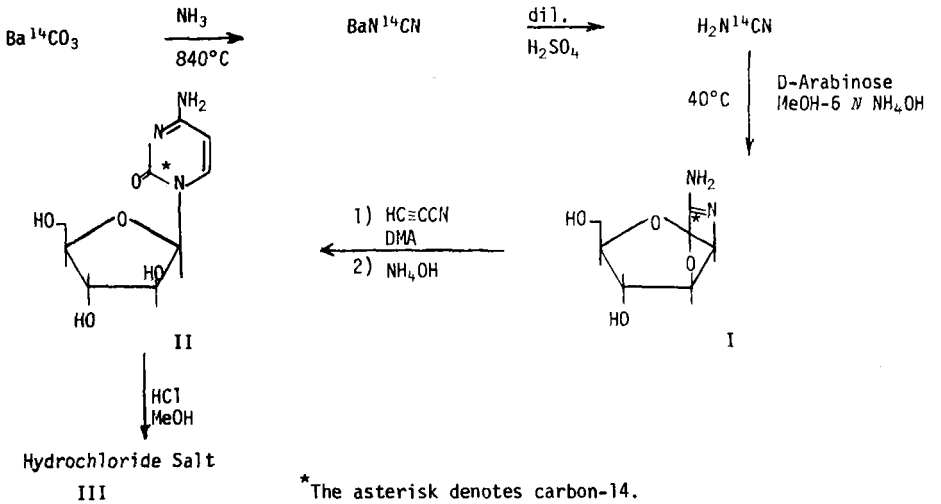
1-β-D-Arabinofuranosylcytosine[1] is an effective agent for the treatment of acute leukemias and lymphomas[2]. It inhibits DNA synthesis[3] and has shown antiviral[4] and antitumor[5] activities. The 5'-(1-adamantoyl) derivative[6] of 1-β-D-arabinofuranosylcytosine has exhibited superior antileukemic[7] and immunosuppressive[8] properties, when compared to the parent compound, in test animals (mice and rats). Carbon-14 labeling of 1-β-D-arabinofuranosylcytosine was carried out to provide radioactive drug for planned absorption and metabolism studies with the drug itself and its adamantoyl and other esters.

DISCUSSION AND RESULTS

The reaction sequence for the synthesis of 1-β-D-arabinofuranosylcytosine-2-^{14}C (II) and its hydrochloride salt (III) is shown in Scheme 1. Barium carbonate-^{14}C was pyrolyzed at 840°C in a stream of ammonia gas to give, in virtually quantitative yield, barium cyanamide-^{14}C. The latter was treated with dilute sulfuric acid to liberate cyanamide-^{14}C according to the procedures of Zbarsky and Fischer[9].

Scheme 1

Reaction Sequence for the Synthesis of
1-β-D-Arabinofuranosylcytosine-2-^{14}C
and Its Hydrochloride Salt



*The asterisk denotes carbon-14.

The procedures for the condensation of D-arabinose with cyanamide to produce 2-amino-β-D-arabinofurano[1',2':4,5]-2-oxazoline-2-^{14}C (I) and the conversion of the latter to 1-β-D-arabinofuranosylcytosine-2-^{14}C were modifications of the procedures of Sanchez and Orgel[10]. The reaction conditions, as

described, proved unsatisfactory on the 5-10 millimole scales required for radioisotopic synthesis.

In the reaction of D-arabinose with cyanamide, close adherence to the described procedure[10] resulted in low yields of 2-amino-β-D-arabinofurano [1',2':4,5]-2-oxazoline-2-^{14}C with low purity. A number of trial runs were carried out in which the ratio of reactants, the solvent system, the reaction time, and the reaction temperature were varied to determine optimum reaction conditions. The results indicated that the molar ratio of cyanamide to D-arabinose could be reduced from 2:1 to 4:3, but no further, without affecting the radiochemical yield and the purity of the product. This is of importance since cyanamide is the label-bearing reactant. MeOH-6*N* NH_4OH (5:1 v/v) was the solvent mixture of choice; MeOH-water (5:1 v/v) gave products of good purity but in lower yields. The reaction temperature must be carefully controlled. The optimum was found to be 40°C. Lower (27°C.) or higher (65°C.) temperatures resulted in products of lower purity and/or in lower yields. Cyanamide must be purified, i.e., any dicyanamide present must be removed, since products contaminated with dicyanamide were difficult to purify without heavy loss.

In the radioactive preparation, 7.5 mmoles of D-arabinose and 10 mmoles of cyanamide-^{14}C were stirred in 2 ml of 5:1 v/v MeOH-6*N* NH_4OH for 24 hours at 40°C to give 47.6% chemical yield (based on cyanamide) of 2-amino-β-D-arabinofurano[1',2':4,5]-2-oxazoline-2-^{14}C.

2-Amino-β-D-arabinofurano[1',2':4,5]-2-oxazoline-2-^{14}C was treated with cyanoacetylene[11] in a two-stage procedure[10] to give 1-β-D-arabinofuranosyl-cytosine-2-^{14}C. In numerous trial runs the reported 80%-90% yield could not be reproduced. The best result was a 32% yield of 1-β-D-arabinofuranosyl-cytosine hydrochloride (III). In the radioactive preparation, the crude

hydrochloride salt was chromatographed on a column of silica gel eluted with 7:3 v/v EtOH-EtOAc. This procedure separated a dark gum from the crystalline product but the latter on recrystallization gave roughly equal amounts of two materials of different melting points but virtually identical R_f values by TLC. One of these two materials proved to be 1-β-D-arabinofuranosylcytosine-2-^{14}C hydrochloride while the other was the free base, 1-β-D-arabinofuranosylcytosine-2-^{14}C, as evidenced by elemental analyses and a negative $AgNO_3$ test. A total of 3.99 mCi of products was obtained, representing 42.5% radiochemical yield from 2-amino-β-D-arabinofurano[1',2':4,5]-2-oxazoline-2-^{14}C. Both products were shown to be radiochemically pure by TLC with two solvent systems: (1) n-BuOH saturated with H_2O and (2) 7:3 v/v EtOH-EtOAc.

EXPERIMENTAL

Melting points were uncorrected. Radioactivity determinations were carried out with the internal standard method.

Barium Cyanamide-^{14}C and Cyanamide-^{14}C

A mixture of 139 mg (40 mCi) of ^{14}C-labeled (from New England Nuclear Corporation) and 875 mg of non-labeled barium carbonate was pyrolyzed at 840°C for 4 hrs under a stream of ammonia gas at 100 ml/min. The resulting rock-like Ba$N^{14}CN$ weighed 897 mg (98.5% yield), of which 851 mg was treated with dilute H_2SO_4 according to the procedure of Zbarsky and Fischer[9] to liberate $H_2N^{14}CN$. The crude material was dissolved in Et_2O and filtered to remove insoluble inorganic salts and/or dicyanamide. Removal of Et_2O gave 168 mg (83% yield) of $H_2N^{14}CN$. This material was repurified immediately prior to use by extraction into Et_2O and removal of the solvent under reduced pressure.

2-Amino-β-D-arabinofurano [1',2':4,5]-2-oxazoline-2-^{14}C (I)

A mixture of 1.126 g (7.5 mmoles) of D-arabinose, 112 mg of $H_2N^{14}CN$ and 310 mg of H_2NCN (total: 10 mmoles), and 2 ml of 5:1 v/v MeOH-6*N* NH_4OH

was stirred under N_2 in an oil bath at 40°C for 24 hrs, then at room temperature for 1 hr and kept in the refrigeartor (0°C) for 19 hrs. The solids were filtered, washed with cold MeOH followed by Et_2O, and dried to give 830 mg of 2-amino-β-D-arabinofurano[1',2':4,5]-2-oxazoline-2-^{14}C, m.p. 180-181°C (dec.), sp. act. 1.99 mCi/mM, 47.6% yield based on $H_2N^{14}CN$.

1-β-D-Arabinofuranosylcytosine-2-^{14}C (II) and its Hydrochloride Salt (III)

A stirred suspension of 819 mg (4.7 mmoles) of 2-amino-β-D-arabinofurano-[1',2':4,5]-2-oxazoline-2-^{14}C in 4.5 ml of DMA was cooled to 10°C under N_2. The cooling bath was removed and 0.35 ml (5.5 mmoles) of cyanoacetylene was added in one portion by means of a syringe. The mixture became warm and after 10 min a wine-red but homogeneous solution resulted. After 30 min, 4.5 ml of *N* NH_4OH was added in one portion. The resulting warm mixture (pH ~9) was stirred for 40 min and concentrated under vacuum (1 mm Hg) at 45°C for 3 hrs. The resulting brown syrup was dissolved in 10 ml of MeOH and 2 ml of 19% w/w HCl-MeOH was added. The mixture (pH 1) was concentrated and the residue was rinsed with Et_2O and dried to give 2.165 g of paste which failed to crystallize as expected. This material was chromatographed on a 52 x 2.8 cm column of silica gel eluted with 7:3 v/v EtOH-EtOAc. After the first 300 ml of eluent (column holdup volume was 430 ml) was collected in one portion, a total of 2.6 l. of eluent was collected in 15 ml fractions at the rate of 1.75 min per fraction. This procedure separated a brown gum from the desired product. Fractions 41 through 166 were pooled and concentrated to give 804 mg solids which were washed with MeOH followed by Et_2O and dried to give 247 mg of white powder, m.p. 213-215°C (dec.), sp. act. 2.00 mCi/mM. Elemental analyses (C, H, N) and a negative $AgNO_3$ test showed this material to be the free base 1-β-D-arabinofuranosylcytosine-2-^{14}C.

Anal. Calcd. for $C_8H_{13}N_3O_5$: C, 44.44; H 5.39; N, 17.28
Found: C, 44.05, H, 5.57; N, 16.92.

The mother liquor of the above material was treated with more HCl-MeOH and from the resulting mixture 1.96 mCi of 1-β-D arabinofuranosylcytosine 2-^{14}C hydrochloride was obtained, m.p. 200-201°C (dec.), sp. act. 2.03 mCi/mM.

Anal.: Calcd. for $C_9H_{14}ClN_3O_5$: C, 38.65, H, 5.05, N, 15.01
Found: C, 38.92, H, 5.11, N, 14.58

The total radiochemical yield was 3.99 mCi or 42.5% based on 2-amino 6-D-arabinofurano[1',2':4,5]-2-oxazoline-2-^{14}C. Both products were radiochemically pure by TLC on silica gel using (1) *n*-BuOH saturated with H_2O and (2) 7:3 v/v EtOH-EtOAc as developing solvent system.

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