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SYNTHESIS OF ^{14}C AND ^{3}H LABELED ARABINOSYL-5-AZACYTOSINE

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SUMMARY

Arabinosyl-[6-^{3}H]5-azacytosine is prepared by catalytic exchange with tritium gas. The preparation of arabinosyl-[2,4-^{14}C]5-azacytosine from barium [^{14}C]carbonate via [U-^{14}C]guanylurea and [2,4-^{14}C]5-azacytosine is described.

Key Words: Arabinosyl-5-azacytosine, ara-AC, 4-amino-1-β-D-arabinofuranosyl-1,3,5-triazine-2(1H)-one, tritium, carbon-14, synthesis

INTRODUCTION

Arabinosyl-5-azacytosine (ara-AC) is a nucleoside antimetabolite which combines the hydrolytic instability of 5-azacytidine and the stereochemical inversion of cytosine arabinoside. This combination of characteristics shows wide therapeutic activity against murine leukemias and some human xenographs.[1] We prepared tritium labeled ara-AC by catalytic exchange to provide material of moderately high specific activity for analytical and pharmacological studies.

Several routes to the preparation of azacytosine nucleosides including the synthesis of [6-^{14}C]5-Azacytidine are reported.[2,3,4] Since the hydrolysis of ara-AC results in the loss of carbon-6 of the triazine ring[5], we elected to adapt the preparation of 5-azacytosine reported by Piskala[6] and of 5-azacytidine reported by Winkley and Robins[7] in order to place the label in the more biologically stable 2,4-positions. The 2,4-carbon-14 labeled compound was a useful complement to the 6-tritium labeled compound in the pharmacological studies.

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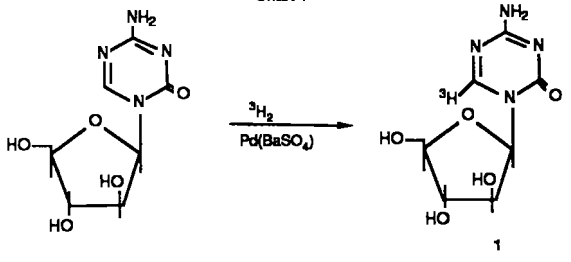
Results and Discussion

Ara-AC was exposed to carrier-free tritium gas in tetrahydrofuran-water solution with palladium on barium sulfate catalyst to give [6-^{3}H]ara-AC(1) as shown in Chart 1. The solubility characteristics of ara-AC and its instability toward hydroxylic solvents demanded special attention to the purification method. After back-exchange with methanol-water to remove labile tritium, the crude 1 was purified by HPLC using a reverse phase C_{18} column

Chart 1



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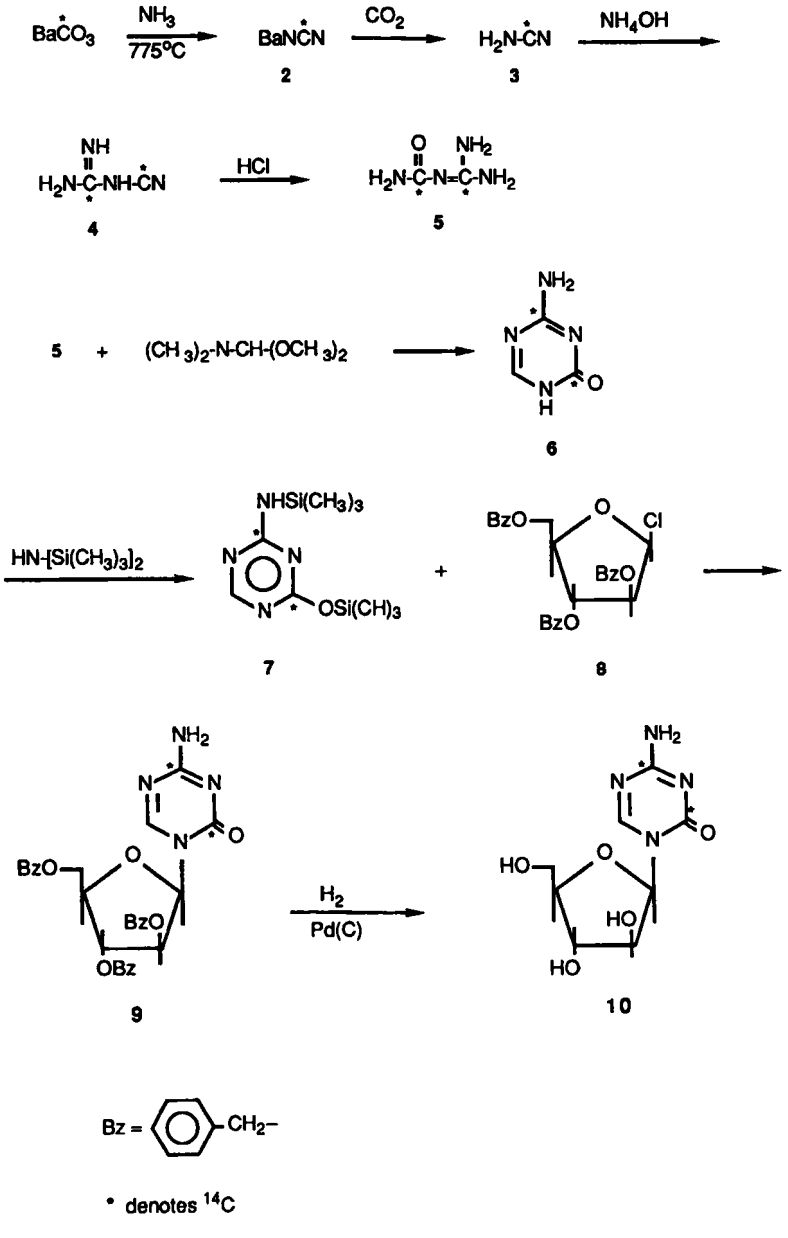
eluted with water. The eluant fraction corresponding to 1 was collected immediately in a flask cooled in dry ice and was lyophilized without letting the sample melt. A yield of 227 mCi of 1 was obtained that was pure by radio-TLC and radio-HPLC. The specific activity was determined by UV absorption at 243 nM and ϵ = 6,800[8] to be 12.5 curies per millimole. The ^{3}H NMR of the product showed a single resonance at δ 8.19 confirming that the label was solely in the 6-position of the triazine ring. Exchange in pH 7 phosphate buffer showed 1% labile tritium and 3% exchange after 24 hours. Stored in dilute ethanol solution at -70°C, compound 1 underwent 10% decomposition in 45 days. A sample stored as a solid at -70°C underwent 10% decomposition in three months. Samples of 1 diluted to a specific activity of 0.1 curies per millimole and stored as a solid at -70°C showed less than 5% decomposition after two years.

The reaction sequence for preparing [2,4-^{14}C]ara-AC (10) is shown in Chart 2. [^{14}C]Cyanoguanidine (4) was prepared from barium [^{14}C]carbonate by heating the carbonate to 775°C in a stream of anhydrous ammonia and treating the product, 2, first with carbon dioxide and then ammonium hydroxide.[9] Unreacted barium [^{14}C]carbonate was recovered in the work-up and reacted again to give a combined 75% radiochemical yield of 4. Compound 4 was quantita-

Chart 2



tively hydrolyzed to [U-^{14}C]guanyl urea (5) with methanolic hydrochloric acid, and 5 was coupled with dimethylformamide dimethylacetal to form [2,4-^{14}C]5-azacytosine (6) in 76% radiochemical yield.[6]

Compound 6 was treated with hexamethyldisilazane, and the resulting silyl derivative (7) was reacted with 2,3,5-tri-O-benzyl-D-ribofuranosyl chloride

(8) to give protected nucleoside 9. The benzyl groups were removed by hydrogenation over palladium catalyst at atmospheric pressure to afford 10. Previous investigators[3,7] found reduction of the 5,6-double bond, and destruction of the triazine ring system as competing side reactions when this hydrogenolysis was carried out at 50 psi. We found no appreciable reduction of the triazine ring at atmospheric pressure. Compound 10 was purified by preparative TLC. The lability of 10 required that the chromatography be done at 0-5°C and that the product be removed from the silica as quickly as possible using chilled solvent. Compound 10 was greater than 98% pure, and was obtained in 3.5% yield from barium [^{14}C]carbonate.

EXPERIMENTAL

Melting points were taken on a Kofler Hot Stage apparatus. ^{3}H NMR spectra were taken on a JEOL FX90Q Fourier transform spectrometer. Ultraviolet spectra were recorded on a Varian model 2290 spectrometer. E. Merck silica gel 60 F-254 and Analtech cellulose analytical plates were used for analytical TLC. Radioactive samples were counted on a Packard Tri-carb 3375 liquid scintillation spectrometer using internal standard in an Omnifluor-toluene 4 g/L cocktail. Developed TLC plates were scanned on a Berthold Model LB 283 Linear Analyzer system. HPLC was done using either a Waters Assoc. Radial Pak C_{18} or an Altex Ultrasphere C_{18} column and a variable wavelength UV-detector and a Berthold Model LB503-HDS radioactivity monitor. Carrier-free tritium gas was purchased from New England Nuclear Corp. Barium [^{14}C]carbonate was purchased from California Bionuclear Corp. Unlabeled ara-AC was supplied by the National Cancer Institute.

4-Amino-1-β-D-arabinofuranosyl-[6-^{3}H]-1,3,5-triazine-2(1H)-one; [6-^{3}H]-ara-AC (1). A 25 mg sample of ara-AC and 25 mg of 5% Pd on $BaSO_4$ were suspended in 0.5 mL of THF-H_2O (8:2). The mixture was exposed to 5 Ci of carrier-free tritium gas for 4 h. The reaction mixture was filtered through a bed of Celite and 3A molecular sieve. The filtrate was diluted with methanol and evaporated to dryness under reduced pressure three times. The product was purified by multiple injections on HPLC using a 4.6 x 250 mm reverse-phase C_{18} 10μ column eluted with H_2O, 6 mL/min. The peaks corresponding to ara-AC

(t_R = 9 min) were collected in a dry ice-cooled container and freeze-dried to give 227 mCi of 1 that was 98% pure by radio-TLC [SiO_2, EtOAc-MeOH-H_2O (4:2:1), R_f 0.58 and cellulose, n-butanol-acetone-H_2O (4:5:1), R_f 0.29] with the same R_f as authentic material. The specific activity of 12.5 Ci/mmol was determined by UV absorption at 243 nm using ϵ 6,800. A sample exchanged in pH 7 phosphate buffer at ambient temperature showed 1% exchange after 1 h and 3% after 24 h. Tritium NMR (DMSO-d_6) δ 8.19 (s, 6-^{3}H) corresponds to δ 8.22 (s) reported for the unlabeled material.[3]

[U-^{14}C]Cyanoguanidine (4).[9] Barium [^{14}C]carbonate (895 mg, 4.5 mmol, 250 mCi) was heated at 760-775°C for 6 h in a stream of anhydrous ammonia. The product was suspended in 2 mL of H_2O, cooled in an ice bath, and excess CO_2 was added. The precipitated $Ba^{14}CO_3$ was removed by centrifugation, and the supernatant was evaporated to give 76.6 mg (40%) of [^{14}C]cyanamide (2). Compound 2 was refluxed in 2 mL of H_2O with 0.05 mL of conc. NH_4OH for 2 h. The volatiles were removed under reduced pressure to give 68.2 mg of 4 with specific activity of 112 mCi/mmol, or 90.7 mCi. Radio-TLC [cellulose F, n-butanol-acetic acid-H_2O (5:2:3)] showed no impurity. The residue from the centrifugation was again reacted as above to yield an additional 95.6 mCi of 4 with specific activity of 68 mCi/mmol.

[U-^{14}C]Guanylurea (5). A mixture of 90.9 mCi (352 mg, 4.2 mmol) of [U-^{14}C]cyanoguanidine in 5 mL of methanol saturated with anhydrous HCl was stirred at 0°C for 10 min and then at ambient temperature for 60 min. The volatiles were removed under reduced pressure, and the residue was dried *in vacuo*. The product was dissolved in 5 mL of H_2O, and excess Bio-Rad AG1-X8 ion-exchange resin in the OH-form was added. The resin was removed by filtration, and the filtrate was evaporated under reduced pressure and dried *in vacuo* to give 573 mg (83.7 mCi, >100% chemical, 92% radiochemical yield) of 5 with specific activity of 146 μCi/mg (14.9 mCi/mmol). Radiochemical purity was 99% by radio-TLC [cellulose F, n-butanol-acetic acid-water (5:2:3), R_f 0.64.

[2,4-^{14}C]5-Azacytosine (6). A mixture of 573 mg (5.6 mmol) of [U-^{14}C]-guanylurea and 0.8 mL (6 mmol) of dimethylformamide dimethylacetal in 5 mL of

methanol was stirred for 20 h at ambient temperature. The volatiles were removed under reduced pressure, and the residue was crystallized from hot water to give 424 mg (64 mCi, 63% chemical, 76.4% radiochemical yield) of 6 with specific activity of 151 μCi/mg (17 mCi/mmol). Radiochemical purity was 92.5% by radio-TLC [cellulose F, n-butanol-acetic acid-water (5:2:3), R_f 0.48].

4-Amino-1-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)-[2,4-^{14}C]-1,3,5-triazine-2(1H)-one (9). A slurry of 64 mCi (424 mg, 3.78 mmol) of [2,4-^{14}C]5-azacytosine and 20 mg of ammonium sulfate in 5 mL of hexamethyldisilizane was refluxed under a N_2 atmosphere for 4.5 h. The volatiles were removed under reduced pressure, and the residue, 7, was dried in vacuo. A solution of 2.278 g (4 mmol) of 2,3,5-tri-O-benzyl-1-O-p-nitrobenzoyl-β-D-arabinofuranose in 18 mL of dry (Al_2O_3) CH_2Cl_2 was cooled to -5°C, and anhydrous HCl was bubbled through the mixture for 2 h. The reaction mixture was warmed to room temperature and filtered through Celite. The filtrate was evaporated under reduced pressure and dried in vacuo to give 8 as a viscous oil. The chloro-sugar, 8, was combined with the disilyl compound, 7, in 8 mL of ethylene dichloride, and the mixture was refluxed for 18 h. The solution was cooled, and 2 mL of methanol was added and stirred for 1 h, and then 1 mL of triethyl-amine was added and stirred for 30 min. The mixture was filtered through Celite, and the filtrate was evaporated to dryness under reduced pressure. The crude 9 was chromatographed on a column of 100 g of silica gel eluted with ethyl acetate. The yield was 1.172 g (43.4 mCi, 65.6% chemical, 67.9% radiochemical yield) of 9 with specific activity of 37.1 μCi/mg (17.5 mCi/-mmol). There was no impurity by radio-TLC (silica gel, ethyl acetate, R_f 0.22).

4-Aminol-β-D-arabinofuranosyl-[2,4-^{14}C]-1,3,5-triazine-2(1H)-one; [2,4,-^{14}C]ara-AC (10). The benzylated nucleoside, 9, (1.172 g) was suspended in 12 mL of ethanol, and 8 mL of methanol that was 2.91 M in anhydrous HCl and 125 mg of 10% Pd on carbon were added. The mixture was exposed to hydrogen at atmospheric pressure and ambient temperature until no further consumption of H_2 was observed (5.5 h). The catalyst was removed by filtration, and the

volatiles were removed under reduced pressure. The residue was redissolved in methanol, and excess triethylamine was added, and the mixture was evaporated and dried in vacuo. The product was stirred for 2 h with 50 mL of $CHCl_3$ and then filtered. The residue was dried in vacuo to give 10 that was 72% pure by radio-TLC.

The impure 10 was applied to a series of preparative silica gel TLC plates using THF-H_2O (8:2) as solvent. The plates were eluted with ethyl acetate-methanol-H_2O (4:2:1) in pre-chilled tanks at 0-5°C. The desired bands were washed from the silica gel with THF-H_2O (8:2), and the solutions of 10 were quickly evaporated under reduced pressure and vacuum dried. The yield was 4.28 mCi with specific activity of 15.5 mCi/mmol; m.p. 215-222°C (dec) [lit[3] 223-225°C (dec)]; UV max (EtOH) 243 nm (ϵ 6516) [lit.[3] 243 nm (ϵ 6800)]. Radiochemical purity was determined by radio-TLC as follows: 98% [silica gel F, ethyl acetate-methanol-water (4:2:1), R_f 0.64 (authentic 10, R_f 0.64)]: 98% [cellulose F, n-butanol-acetone-water (4:5:1), R_f 0.22 (authentic 10, R_f 0.22] and by HPLC: 98% [Waters Radial Pak RPC_{18}, water, 2 mL/min, UV-210 nm and radioactivity detectors, R_t 10.2 min (authentic 10, R_t 10.2 min)].

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