SYNTHESIS OF [8-^{3}H]PENTOSTATIN

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SUMMARY

Starting from 3-(2-deoxy-β-D-erythro-pentofuranosyl)-6,7-dihydroimidazo[4,5-d][1,3]diazepin-8(3H)-one (4), the synthesis of both the R-and S-isomers of [8-^{3}H]pentostatin has been achieved. The separation of the desired R-isomer in ca. 24% overall chemical yield from the R,S-mixture was effected by preparative reverse-phase chromatography utilizing a C_{18} stationary support. Crystalline product of high specific activity (227 mCi/mmole) was obtained.

Key Words: Adenosine deaminase inhibitor, [8-^{3}H]pentostatin, preparative reverse-phase chromatography.

INTRODUCTION

There is currently a burgeoning interest in pentostatin (1)[1], a nucleoside of unusual structure derived initially from the fermentation beers of Streptomyces antibioticus NRRL 3238[2] and more recently by total synthesis.[3] This along with its close structural relative coformycin (2)[4] represents the most potent inhibitor known of adenosine deaminase (ADA), the enzyme responsible for the N-6 deamination of adenine nucleosides. In specially devised kinetic experiments[5], 1 showed a K_i=2.5 x 10^{-12} M and 2 a K_i=1.0 x 10^{-11} M against human erythrocytic ADA, thus qualifying both as essentially irreversible inhibitors. While pentostatin has proven quite effective as a co-drug for enhancing the antiviral[6] and antitumor[7] efficacy of various adenine nucleosides both in vitro and in vivo, it alone is being

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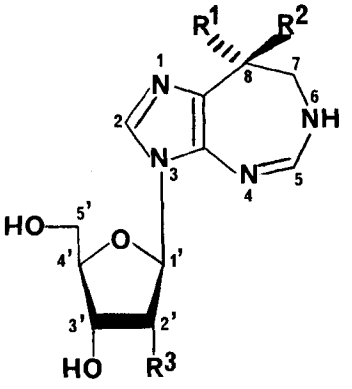
accorded increasing attention as an agent for the inhibition of lymphocytic ADA. Such a therapy would be desirable in the treatment of lymphoid malignancies[8] and in immunosuppression.[9]

In order to study the tissue distribution and metabolic fate of pentostatin *in vivo*, Glazko *et al.*[10] synthesized ^{3}H(G)-labeled 1 via tritium exchange and examined its incorporation into rat tissue following i.m. injection. Various investigators[11] have reported the use of microbiologic and enzymatic assays for the acquisition of similar information in other animal species.

With the necessity to carry out further metabolic studies *in vivo* and possible mechanism of action studies *in vitro* with pentostatin, we report here a synthesis of [8-^{3}H]pentostatin, 3, of high specific activity in which tritium is appended specifically to C-8. Such a synthesis via [^{3}H]$NaBH_4$ reduction of readily available ketonucleoside 4 was facilitated by the recent disclosure of novel synthetic methodology from these laboratories[3] that enables one in principle to generate pentostatin labeled with ^{3}H or ^{14}C (or corresponding stable isotopes) at various specified positions. We chose the former isotope because it required only a single-step synthetic operation.

Reduction of 4 under standard conditions was carried out initially with a small amount of unlabeled $NaBH_4$ to react with any active hydrogen present and to raise the solution pH. This minimized loss of tritium since $NaBH_4$ hydrolyzes much more slowly at elevated pH. We then treated the mixture with less than a theoretical amount of [^{3}H]$NaBH_4$ over a long reaction period in order to insure maximum incorporation of tritium[12] and hence minimize possible tritium loss due to an isotope effect. Sodium borohydride was added at the end of the reaction to drive it to completion and to facilitate the isolation of the mixture of R,S-isomers, 3 and 5.



1: R^1=OH; R^2=R^3=H

2: R^1=R^3=OH; R^2=H

3: R^1=OH; R^2=^{3}H; R^3=H

4. R^1,R^2=O; R^3=H

5. R^1=^{3}H; R^2=OH; R^3=H

In order to effect a clean separation of the crude, water-soluble R,S-[8-^{3}H]pentostatin mixture, we developed a reverse-phase variant of a glass column preparative chromatographic procedure reported by Still.[13] Similar preparative procedures utilizing a modified silica gel[14] or micronized XAD-2 resin[15] as the stationary support have been reported. Employing an octadecylsilyl-derivitized silica gel stationary support, we were able to effect nearly complete separation of R- and S-isomers, 3 and 5, respectively. Subsequent crystallization of the crude R-isomer afforded desired 3 of high specific activity (227 mCi/mmole). Physical characterization showed a chemical purity of $\geq$ 98% by hplc[16] and UV spectroscopy[17] and a radiochemical purity of $\geq$ 98% by hplc utilizing liquid scintillation spectroscopy for assay.

EXPERIMENTAL

Radioactive samples were counted in Packard Tri-Carb liquid scintillation spectrometers, Models 3375 and 3003, using either Omnifluortoluene (4 g/L) or Amersham PCS cocktails. E. Merck silical gel 60 F-254 analytical plates were used for tlc. Developed tlc plates were scanned on a Varian Berthold Radioscanner fitted with a Model LB 242 x dual ratemeter. UV spectra were taken on a Cary Model 118c spectrophotometer. [^{3}H]$NaBH_4$ was purchased from New England Nuclear Corporation.

[^{3}H]$NaBH_4$ Reduction of 3-(2-deoxy-β-D-erythro-pentofuranosyl)-6,7-dihydroimidazo[4,5-d][1,3]diazepin-8(3H)-one 4

A solution of 1.064 g (3.8 mmol) of the ketonucleoside (4), hydrated with 0.8 mole equivalent of H_2O, in 11 ml MeOH and 6 ml H_2O was cooled to 0°C, 12.5 mg (0.3 mmol) of unlabeled $NaBH_4$ was added, and the mixture stirred for 2 hr at 0°C. The reaction vessel was cooled in liquid N_2 and 1.5 Ci (4.95 mg, 0.13 mmol) of [^{3}H]$NaBH_4$ (2.88 Ci/mmol hydride equivalent) in 1.0 ml EtOH was added. The reaction vessel was attached to a vacuum manifold and evacuated. The reaction was closed from the manifold, and the mixture stirred at 0°C for 2 hr and then at room temperature for 18 hr. The reaction mixture was frozen in liquid N_2 and the gases above it were transferred via a Toepler pump for disposal. The reaction flask was removed from the manifold, and 40.6 mg (1.07 mmol) of unlabeled $NaBH_4$ was added. With this addition a total of 1.5 mmol of labeled and unlabeled $NaBH_4$ had been added, which corresponded to a specific activity of 250 mCi/mmol of hydride equivalent. The sides of the flask were washed down with 0.5 ml EtOH, then the flask was replaced on the manifold, frozen in liquid N_2, and evacuated. The reaction mixture was stirred at 0°C for 2 hr and then at room temperature for 3 hr. The mixture was frozen in liquid N_2 and the gases transferred for disposal. Glacial AcOH (262 μl, 6 mmol) was added to the frozen mixture which was then attached to the manifold and evacuated. After allowing the mixture to come to room temperature, and reaction had ceased, the gases and then the solvent were transferred under vacuum for disposal as radioactive waste.

The residue was dissolved in 7 ml of H_2O and the pH adjusted to 7.48 with dilute AcOH. Analysis of this solution by tlc (CH_3CN-0.2 N aqueous NH_4Cl, 3:1) indicated the absence of the starting ketone (R_f 0.35) and a single

radioactive component with an R_f (0.2) identical to that of an authentic sample of pentostatin. After storage overnight at 0°C, the solvent was removed by lyophilization to give 1.50 g (813 mCi, 54% radiochemical yield) of crude R,S-[8-^{3}H]pentostatin as a gum. Hplc analysis showed a pattern of two peaks of equal intensity, identical to that of synthetic R,S-pentostatin.[16]

Separation of R,S-isomers by Preparative Reverse-phase Chromatography and Crystallization of (R)-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3,6,7,8-[8-^{3}H]tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol (3)

To a glass column (8x 70 cm) fitted with a fritted disc, large-bore teflon stopcock, and 24/40 upper ground glass joint was added ca. 250 g of Waters preparative, reverse-phase octadecylsilyl-derivitized silica gel slurried in mobile phase (5% MeOH in H_2O, pH 7.5). The support material was packed to a depth of 12-15 cm under 4 psi N_2 pressure followed by elution with ca. 1 ℓ of mobile phase. The crude tritiated mixture dissolved in 7 ml H_2O was transferred by Pasteur pipette to the column with care taken not to disturb the surface of the support material. The sample container was rinsed 3x with mobile phase and the rinsings were added to the column. The sample and rinsings were introduced onto the column support under 4 psi N_2 pressure. The sides of the column were rinsed 5x with mobile phase and the combined rinsings introduced as above. The column was carefully filled with ca. 2 ℓ mobile phase to separate the isomers. A flow rate of 50-100 ml/min was obtained by applying 4 psi N_2 pressure to the column head. Twenty-eight 100-150 ml fractions were collected over 45 min. After assay by hplc, the appropriate fractions were combined into three lots. Lot 1 contained S-[8-^{3}H]pentostatin, lot 2 a mixture of isomers, and lot 3 predominately R-[8-^{3}H]pentostatin. The

solvent was evaporated from the third lot by rotary evaporation (35°C/5 mm) to leave a pale-yellow syrup. Lots 1 and 2 were evaporated as above and stored in aqueous MeOH at 3°C.

The syrup from lot 3 was transferred to a 10 ml beaker using 0.5 ml of H_2O (85°C, pH 7.5) and minimal MeOH. The MeOH was evaporated under a steady stream of N_2 to give a mushy crystalline mass which solubilized upon heating to 85°C. Hot MeOH (5 ml) was added with thorough stirring and the solution was allowed to cool to room temperature during which crystallization began. The solution was kept at 3°C for 3 da. The crystals were collected by filtration, washed with 5 ml cold MeOH, air dried for 30 min, and then dried overnight (25°C/100 mm). Light beige crystals (246.01 mg, 24% yield) with a specific activity of 227 mCi/mmole were obtained. The sample showed a chemical purity of $\geq$ 98% by hplc and UV spectroscopy and a radiochemical purity of $\geq$ 98% by hplc using liquid scintillation spectroscopy for assay.

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16. EM Reagents LiChrosorb RP-18, 10 um; 250 mm x 4.6 mm analytical column; flow rate 1.3 ml/min; mobile phase 10% MeOH in 0.005 M aqueous $(NH_4)_2HPO_4$. Retention time S-isomer = 4 min; R-isomer = 5.5 min.

17. λ_{max} 282 (Σ 8052) in pH 7 buffer.