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Synthesis and Characterization of [Phenyl-^{3}H] Clonidine Hydrochloride at High Specific Activity

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SUMMARY

p-Aminoclonidine hydrochloride **1** was converted to m-dibromo-p-iodoclonidine **3** and the latter was used as a precursor to prepare [phenyl-^{3}H] clonidine hydrochloride **4** at high specific activity *via* a selective catalytic dehalogenation with tritium.

Key Words: Clonidine, Selective Dehalogenation, Tritium, Tritium NMR

INTRODUCTION

Clonidine is an antihypertensive drug whose pharmacological activity is thought to reside in its interaction with the central alpha-2 adrenergic receptor (1) and also more recently what has been described as the imidazoline receptor (2). Previously, clonidine has been radiolabelled with ^{14}C (3-5) and ^{3}H (5) at low specific activity. To improve the utility of [^{3}H] clonidine as a radioligand we endeavored to significantly increase its specific activity.

DISCUSSION

As a suitable precursor we chose to prepare a polyhalo clonidine derivative. We had earlier employed this same strategy in an attempt to prepare [^{3}H] strychnine at high specific activity but realized only marginal success due to a competing side reaction of over-reduction (6). However, we had reason to believe that in the case of clonidine a selective catalytic tritium dehalogenation could be achieved. As shown in Scheme 1, bromination of p-aminoclonidine hydrochloride **1** afforded m-dibromo-p-aminoclonidine hydrobromide **2** in high

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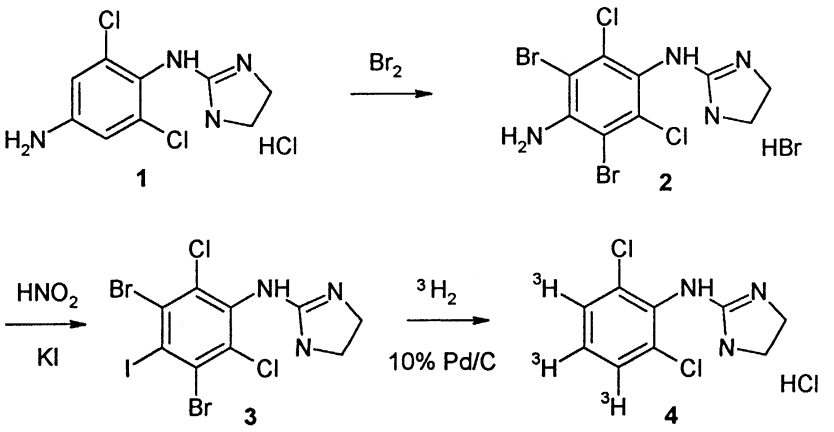
yield. Encouraged by related literature precedent (7), intermediate **2** was diazotized and treated with potassium iodide to obtain m-dibromo-p-iodoclonidine **3** in good yield.

Precursor **3** could be catalytically reduced by tritium with good selectivity to afford [phenyl-^{3}H] clonidine hydrochloride **4.** Radioligand **4** was preliminarily purified by TLC followed by preparative HPLC. It was found to be homogeneous on TLC and HPLC and co-chromatographed with authentic standard. Over the course of many runs the specific activity range observed in the preparation of **4** was measured by UV and mass spectral analysis to be 45 – 63 Ci/mmol (8). A proton decoupled tritium NMR of **4** (Figure 1) showed a multiplet at δ 7.50 ppm indicating exclusive aromatic tritium labelling. Analogous attempts to prepare the corresponding triiodoclonidine *via* m-diiodo-p-aminoclonidine (9) were not as successful.

Use of this radioligand has facilitated understanding of the alpha-2 adrenergic receptor (10,11).

Scheme 1



EXPERIMENTAL

Evaporations were carried out on a Buchi rotary evaporator *in vacuo* at bath temperatures less than 40°C. TLC was performed on Analtech plates coated with silica gel. Autoradiography was performed at 0°C after spraying with 2,5-diphenyloxazole and exposing the plates to x-ray film. TLC plates were also scanned (~ 3 min) for radioactivity (~ 10 μCi). Preparative and analytical HPLC were performed with peak detection done simultaneously by UV (280 nm) and a liquid scintillation flow monitor. The proton and tritium NMR spectra were recorded on a Bruker 200 mHz spectrometer and chemical shifts are reported in parts per million (ppm) downfield from internal TMS. High resolution mass spectra were obtained from Shrader Analytical Laboratories (Detroit, Michigan).

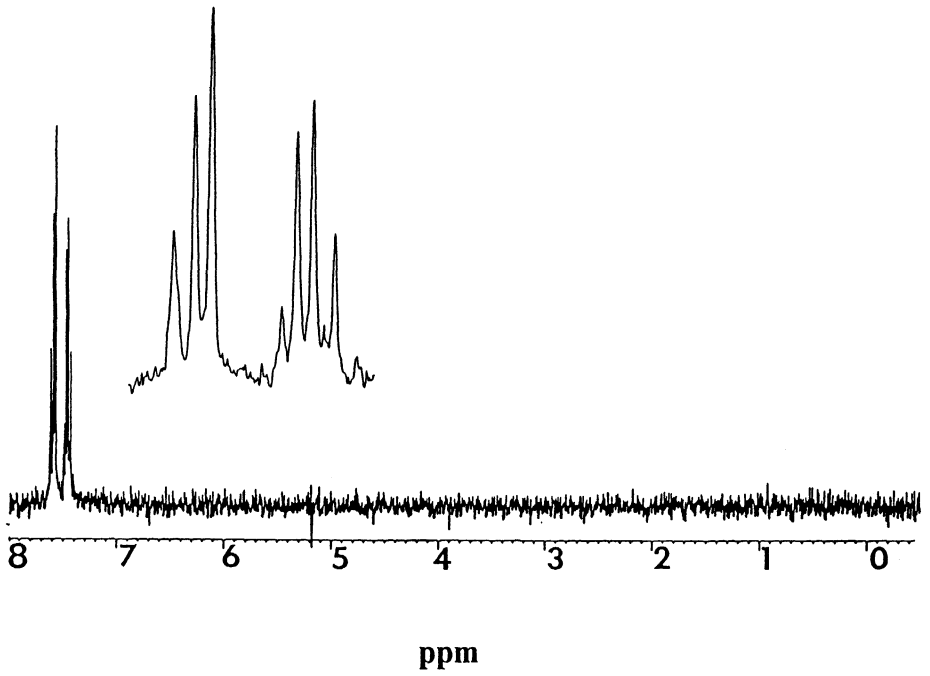


Figure 1: Proton decoupled ^{3}H NMR (CD_3OD) of **4.**

m-Dibromo-p-aminoclonidine Hydrobromide (2) To a solution of 100 mg (0.36 mmol) of p-aminoclonidine hydrochloride **1** (RBI Cat. # B2) in 8 mL of acetic acid with 2 mL of trifluoroacetic acid was added 172 mg (1.07 mmol) of bromine in 2.9 mL of acetic acid with rapid stirring at room temperature. About halfway through the addition a yellow precipitate appeared. The reaction was stirred at room temperature for 4 h and then excess solvent was removed at reduced pressure to afford 170 mg (100 % yield) of m-dibromo-p-aminoclonidine hydrobromide **2** as a light yellow solid (mp > 300^{0}C, darkens at 200^{0}C), that was homogeneous on silica gel TLC (ethyl acetate:ethanol:diethylamine (90:10:8)) and HPLC (reverse phase eluted with acetonitrile:0.01 N potassium phosphate (pH 7.5) (60:40)); ^{1}H NMR (CD_3OD) δ 3.80 ppm (broad s); ^{13}C NMR (CD_3OD) δ 160.83, 147.93, 136.21, 120.48, 107.33 and 44.37 ppm; IR (KBr) 3300, 3140, 1655, 1610, 1440 and 1380 cm^{-1}; UV (ethanol) ε_{318} = 3441, ε_{252} = 8987. High Resolution Mass Spectrum: Calc'd for $C_9H_8N_4Br_2Cl_2$: 401.8470; Found: 401.8466.

m-Dibromo-p-iodoclonidine (3). To a slurry of 150 mg (0.31 mmol) of **2** in 40 mL of 2N hydrochloric acid at room temperature was added 27 mg (0.39 mmol) of sodium nitrite in 0.7 mL of water dropwise with rapid stirring. The reaction was stirred an additional 20 min at room temperature (positive starch iodide test) and then 4 g of potassium iodide in 4 mL of water was added. The solution was heated at 80-100^{0}C for 2 h and nitrogen bubbling was noted. The reaction was

then allowed to stir at room temperature overnight. After this time it was cooled in an ice bath and made basic to pH 8 with concentrated ammonium hydroxide and extracted with five 20 mL portions of chloroform. Evaporation of the chloroform layer yielded 280 mg of a residue that was purified by preparative TLC on three 1000 μ silica gel plates eluted with benzene:ethyl acetate:diethylamine (7:2:1). The main band was visualized by UV (Rf = 0.65), scraped and eluted with 10% methanol in chloroform. Evaporation of the eluent afforded 123 mg (78% yield) of m-dibromo-p-iodoclonidine **3** as a tan solid that was homogeneous on silica gel TLC (benzene:ethyl acetate:diethylamine (7:2:1)) and 95% pure by HPLC (reverse phase eluted with acetonitrile:0.01 N potassium phosphate (pH 7.5) (60:40)); ^{1}H NMR (CD_3OD) δ 3.50 ppm (s); ^{13}C NMR (DMSO d6) δ 156.88, 147.76, 128.64, 128.55, 101.41 and 41.23 ppm. IR (KBr) 2940, 1650 cm^{-1}; UV (ethanol) ε_{258} = 9644. High Resolution Mass Spectrum: Calc'd for $C_9H_6N_3Br_2Cl_2I$: 510.7349. Found: 510.7340. A small amount of what is likely m-dibromoclonidine was also observed: Calc'd for $C_9H_7N_3Br_2Cl_2$: 384.8382; Found: 384.8384.

[Phenyl-^{3}H] Clonidine Hydrochloride (4). A solution of 25 mg (0.05 mmol) of precursor **3** in 10 mL of ethanol with 150 mg of 10% palladium on charcoal was reduced with 100 Ci of tritium gas at room temperature for 4 h with rapid stirring. After this time, labile tritium was removed by several methanol evaporations and following catalyst filtration, the crude product was stored in 10 mL of methanol (total radioactivity = 1.24 Ci). It was purified on two 1000 μ silica gel TLC plates developed with chloroform:methanol:ammonia (20:1:0.1) and unlabelled clonidine hydrochloride (RBI Cat. # B-001) was allowed to migrate in a separate side lane to facilitate product location by UV visualization. After plate development the desired product (Rf = 0.5) was scraped from the plate and eluted with ethanol to afford 328 mCi of product **4**. Final purification of the radioligand was accomplished by HPLC on a reverse phase column eluted with water:acetonitrile:diethylamine (90:10:0.1) and the purest fractions were combined. The mobile phase was removed by rotary evaporation and the product was dissolved in ethanol:water (7:3). Typically, 328 mCi of TLC purified product afforded 118 mCi (a 5 % radiochemical yield based on **3**) of purified **4** that was found to be 98% radiochemically pure on silica gel TLC (chloroform:methanol:ammonia (20:1:0.1)) and HPLC (reverse phase eluted with acetonitrile:0.01 N potassium phosphate (pH 7.5) (40:60)). Also, in these chromatographic systems radioligand **4** co-chromatographed with authentic clonidine hydrochloride. The specific activity of **4** was determined to be 45 Ci/mmol by UV spectroscopy (ethanol) where ε_{271} = 528 for cold clonidine hydrochloride and the UV spectrum of **4** was also superimposable on that of cold clonidine. A proton decoupled tritium NMR (Figure 1) showed a multiplet at δ 7.50 ppm.

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