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THE PREPARATION AND CHARACTERIZATION OF (±)-[PHENOXY-^{3}H(N)] PHENOXYBENZAMINE HYDROCHLORIDE AT HIGH SPECIFIC ACTIVITY[1]

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SUMMARY

(±)-Phenoxybenzamine is a ligand capable of irreversibly alkylating the alpha-1 adrenergic receptor. Previous attempts to radiolabel it have achieved only low specific activities which impeded their use in neurochemical investigations. (±)-[Phenoxy-^{3}H(N)] Phenoxybenzamine.HCl (1) has been prepared in our laboratory by the catalytic reductive aryl debromination of an appropriate brominated precursor with tritium gas. ^{3}H NMR showed exclusive radiolabeling on the phenoxy ring and the radioligand had a specific activity of 32.4 Ci/mmol.

Key Words: (±)-phenoxybenzamine.HCl, alpha-1 adrenergic receptor, tritium, ^{3}H NMR.

INTRODUCTION

Present in a wide variety of tissues, the alpha-1 adrenergic receptor is a key component in the autonomic and central nervous system. (±)-Phenoxybenzamine.HCl is an alpha-1 adrenergic antagonist possessing a reactive β-chloroethylamine side chain capable of alkylating the receptor.[2] Previously, (±)-phenoxybenzamine.HCl has been isotopically labeled with ^{14}C[3], ^{15}N[4] and ^{3}H[5-12]. Unfortunately, these analogues were all too low in specific activity to allow for the molecular identification of the alpha-1 adrenergic receptor. For this reason, we now disclose our work leading to the preparation of (±)-[phenoxy-^{3}H(N)] phenoxybenzamine.HCl(1) at high specific activity employing ^{3}H NMR to confirm radiolabeling specificity.

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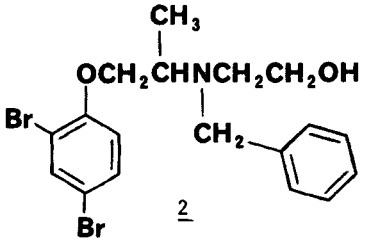
DISCUSSION

The most attractive and straightforward method for the synthesis of 1 appeared to be the reductive tritiation of dibromo intermediate 2. This approach was soon abandoned because of the unavoidable concomitant catalytic debenzylation of 2. Therefore, an alternative and successful strategy for the preparation of 1 was developed and is shown in Figure 1.



2

(±)-1-(2,4-Dibromophenoxy)-2-chloropropane (3) was prepared in 97% yield by the bromination of (±)-1-phenoxy-2-chloropropane. Treatment of 3 with ethanolamine afforded (±)-1-(2,4-dibromophenoxy)-2- (2-hydroxyethylamino) propane (4) in 69.3% yield. It is interesting to note that 4 could not be as easily prepared by the bromination of (±)-1-phenoxy-2-(2-hydroxyethylamino) propane. Precursor 4 was catalytically tritiated with 10% Pd/Al_2O_3 in dioxane to yield (±)-[phenoxy-^{3}H(N)]-1-phenoxy-2-(2-hydroxyethylamino) propane (5) in 61.7% radiochemical yield based on cold precursor 4. Reaction of 5 with benzyl bromide gave (±)-[phenoxy-^{3}H(N)]-1-phenoxy-2-(benzyl-2-hydroxyethylamino) propane (6) in 30% radiochemical yield based on 5 and treatment of 6 with excess $SOCl_2$ afforded (±)-[phenoxy-^{3}H(N)] phenoxybenzamine·HCl (1) in 46.7% radiochemical yield based on 6. The specific activity of 1 was determined to be 32.4 Ci/mmol and its ^{3}H NMR (CD_3OD) afforded a single peak at δ7.05 ppm indicative of exclusive aromatic ring radiolabeling. Use of 1 at such high specific activity has prompted greater insight and understanding of the alpha adrenergic receptor at the molecular level. [13-15]

EXPERIMENTAL PROCEDURES

All chemicals were used as obtained from the manufacturer. Evaporations were carried out on a Buchi rotary evaporator *in vacuo* at bath temperatures below 40°C. Both analytical and preparative TLC were performed on Analtech

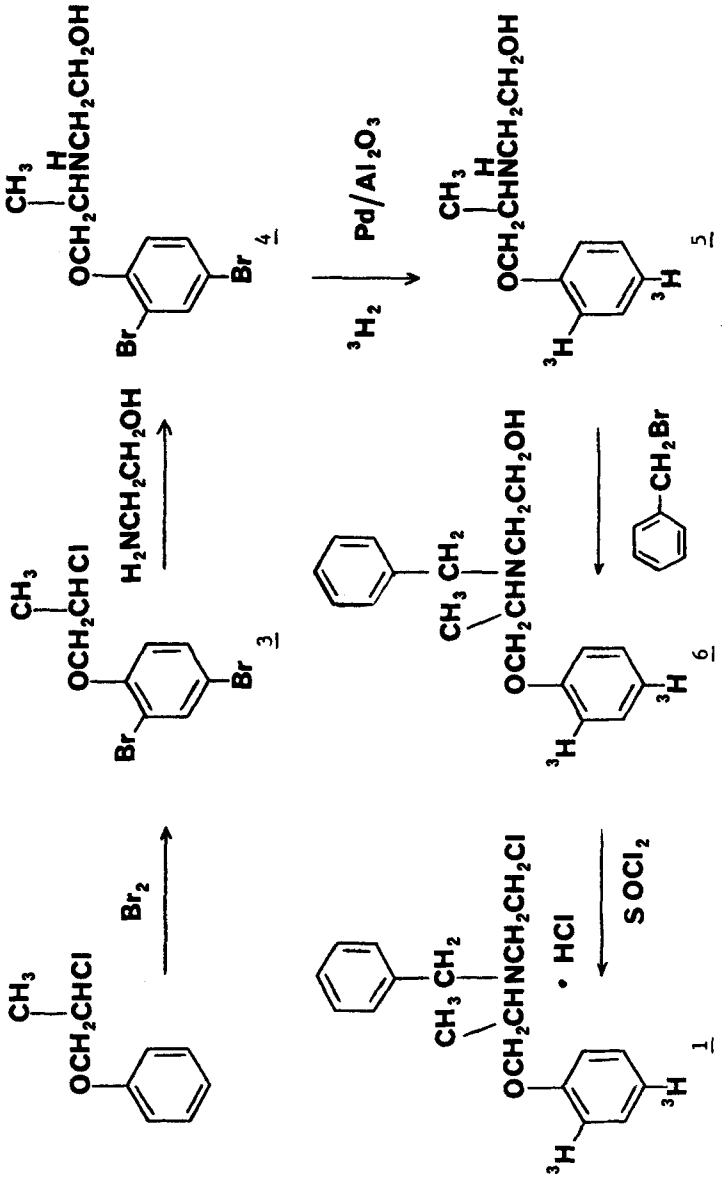


Figure 1

plates. Autoradiography was performed at 0°C after spraying the TLC plates with PPO (DuPont, NEN Research Products) and exposure to Eastman Kodak SB-5 film. TLC plates were also scanned for activity using a Packard 7201 scanner. Analytical HPLC was performed on a Waters HPLC instrument, and peak detection was performed simultaneously using a Waters 440 UV detector and a radioactivity flow monitor detector. Tritium was counted using a Packard 460 C instrument. The ^{1}H, ^{3}H, and ^{13}C NMR spectra were obtained on a Bruker WP 200 mHz NMR instrument and chemical shifts are expressed in parts per million (ppm) downfield from internal $(CH_3)_4$ Si. UV spectra were measured on a Beckman Model 25 spectrophotometer and the IR spectrum was measured on a Perkin-Elmer Model 700 spectrophotometer. The high resolution mass spectrum was performed by Shrader Analytical Laboratories (Detroit, MI).

(±)-1-(2,4-Dibromophenoxy)-2-chloropropane (3). A solution of 450 mg (2.65 mmol) of (±)-1-phenoxy-2-chloropropane (Tokyo Kasei cat # p 117) and 2.0 g (12.5 mmol) of bromine in 5 mL of HOAc was heated and stirred under nitrogen for 4 days at 60°C. After this time rotary evaporation of excess solvent afforded 840 mg (97%) of (±)-1-(2,4-dibromophenoxy)-2-chloropropane that was homogeneous on TLC (reverse phase - CH_3OH : CH_3CN (9:1), Rf 0.7); ^{1}H NMR ($CDCl_3$) δ 7.67 (d, 1, J = 1 Hz), 7.33 (dd, 1, J = 1, 10 Hz), 6.75 (d, 1, J = 10 Hz), 4.40 - 3.95 (m, 3) and 1.68 ppm (d, 3, J = 8 Hz); ^{13}C NMR ($CDCl_3$) δ 154.13, 135.73, 131.28, 114.22, 113.90, 113.42, 74.10, 53.65 and 21.82 ppm. Less stringent bromination conditions afforded only the monobromoprecursor (±)-1-(4-bromophenoxy)-2-chloropropane (Rf 0.8 in the above TLC system).

(±)-1-(2,4-Dibromophenoxy)-2-(2-hydroxyethylamino) propane (4). A solution of 154 mg (0.47 mmol) of (±)-1-(2,4-dibromophenoxy)-2- chloropropane (3) with 1 mL (1.01 g, 16.4 mmol) of ethanolamine in 3 mL of dioxane was heated and stirred under nitrogen for 7 days at 95°C. After this time, the reaction was diluted with $CHCl_3$ and extracted with H_2O to remove excess ethanolamine. The $CHCl_3$ layer was evaporated to yield a residue which was preparatively chromatographed on four 20 x 20 cm 1000 μ silica gel GF plates developed with $CHCl_3$: CH_3OH:

NH_4OH (10: 1: 0.1). The main band was visualized by UV and eluted with EtOH to give 115 mg (69.3% yield) of (±)-1-(2,4-dibromophenoxy)-2-(2-hydroxyethylamino) propane (4) as an oil that was homogeneous on TLC (silica gel - $CHCl_3$: CH_3OH: NH_4OH (10: 1: 0.1) Rf 0.4); ^{1}H NMR ($CDCl_3$) δ 7.65 (d, 1, J = 1 Hz), 7.33 (dd, 1, J = 1, 10 Hz), 6.75 (d, 1, J = 10 Hz), 3.90 (m, 2), 3.67 (m, 2), 3.15 (m, 1), 2.83 (m, 2) and 1.20 ppm (d, 3, J = 8 Hz); ^{13}C NMR ($CDCl_3$) δ 154.13, 135.56, 131.31, 114.59, 113.33, 73.16, 67.99, 61.89, 52.19, 48.11 and 17.52 ppm; IR (NaCl) 2900 (broad), 1610, 1490, 1375 cm^{-1}.

High Resolution Mass Spectrum: Calcd for $C_{10}H_{12}NOBr_2$ (M^+-CH_2OH) 321.9264. Found: 321.9250. No molecular ion was seen but this is common for aminoalcohols.

(±)-[Phenoxy-^{3}H(N)]-1-Phenoxy-2-(2-hydroxyethylamino) propane (5). A solution of 35.4 mg (0. 1 mmol) of (±)-1-(2,4-dibromophenoxy)-2-(2-hydroxyethylamino) propane (4) with 35 mg of 10% Pd/Al_2O_3 in 5 mL of dioxane and 30 μL of Et_3N was reduced for 3h with 80 Ci of tritium gas. After catalyst filtration and labile tritium was removed by EtOH evaporation, the crude product was packaged in 10 mL of EtOH. It was concentrated by rotary evaporation to 300 μL and preparatively chromatograped on two 20 x 20 cm 1000 μ silica gel GF plates developed with EtOAc: CH_3OH: NH_4OH (99: 1: 0.1). The main band was visualized by UV, scraped and eluted with EtOH to afford 2000 mCi (61.7% radiochemical yield based on 4) of (±)-[phenoxy-^{3}H(N)]-1-phenoxy-2-(2-hydroxyethylamino) propane (5) which was homogeneous on silica gel TLC ($CHCl_3$: CH_3OH: NH_4OH (9: 1: 0.1), Rf 0.3 and EtOAc: CH_3OH: NH_4OH (99: 1: 0.1), Rf 0.2). Also, 5 cochromatographed with authentic cold standard in these aforementioned TLC systems (the cold standard was prepared by the treatment of (±)-1-phenoxy-2-chloropropane with ethanolamine; ^{1}H NMR ($CDCl_3$) δ7.27 (m, 2) 6.90 (m, 3), 3.90 (m, 2), 3.65 (t, 2), 3.10 (m, 1), 2.83 (m, 2) and 1.17 ppm (d, 3)). The UV (EtOH) spectrum of 5 was superimposable on that of cold standard and a specific activity of 32.4 Ci/mmol was calculated for it by UV (EtOH) spectroscopy (where ε_{270} = 1299 for authentic cold standard).

(±)-[Phenoxy-^{3}H(N)]-1-Phenoxy-2-(benzyl-2-hydroxyethylamino) propane (6). A solution of 2000 mCi (0.062 mmol) of (±)-[phenoxy-^{3}H(N)]-1-phenoxy-2-(2-hydroxyethylamino) propane (5) and 0.12 mmol of benzylbromide with 13 mg of $NaHCO_3$ in 4 mL of EtOH was stirred and heated at 70°C for 16 h under nitrogen. After this time the reaction was cooled and concentrated to 300 µL by rotary evaporation and crude product 6 was preparatively chromatographed on two 20 x 20 cm 500 µ silica gel GF plates developed with EtOAc: CH_3OH (95: 5), Rf 0.8. It was found advantageous to further purify 6 by HPLC using two µC_{18} columns in tandem eluted with EtOH and a trace of HCl (4 drops of conc. HCl in 1 liter of EtOH) at 0.5 mL/min. Injections of 5 - 10 mCi each were made in 20 - 30 µL of EtOH and a main peak (retention time = 11 min) was collected. Typically, the radiochemical yield of HPLC purified 6 was 30% (based on 5). Intermediate 6 was found to be homogeneous in the aforementioned TLC and HPLC systems and cochromatographed with authentic cold standard (the cold standard was prepared by the hydrolysis of (±)-phenoxybenzamine·HCl (Tokyo Kasei cat # D 158) using NaOH in EtOH at 55°C; ^{1}H NMR ($CDCl_3$) δ 7.40 - 6.80 (m, 10), 4.05 - 3.25 (m, 7), 2.90 - 2.60 (m, 2) and 1.15 ppm (d, 2); ^{13}C NMR ($CDCl_3$) δ 158.67, 139.81, 129.48, 128.61, 128.44, 127.14, 120.90, 114.60, 69.76, 58.88, 54.80, 53.62, 51.34 and 11.98 ppm). Also the UV (EtOH) spectrum of the tritiated intermediate was superimposable on that of cold standard and a specific activity of 32.4 Ci/mmol was calculated for it by UV (EtOH) spectroscopy where ε_{272} = 1578 for cold standard.

(±)-[Phenoxy-^{3}H(N)] Phenoxybenzamine HCl (1). An EtOH solution of 30 mCi of HPLC purified 6 was evaporated to dryness and the residue was taken up in 1.5 mL of $CHCl_3$. HCl gas was bubbled through the $CHCl_3$ solution briefly and then 50 µL (81.5 mg, 0.7 mmol) of $SOCl_2$ was added via syringe. The solution was then stirred at 60°C for 2h. After this time excess solvent was evaporated and the residue was preparatively chromatographed on a single 5 x 20 cm 250 µ $KC_{18}F$ plate developed with CH_3OH: CH_3CN (9: 1). The main band (Rf 0.6 (unreacted 6 ran as a higher band of Rf 0.7)) was visualized by UV, scraped and eluted with EtOH to afford 14 mCi (46.7% radiochemical yield) of 1 (enough aq. HCl was added

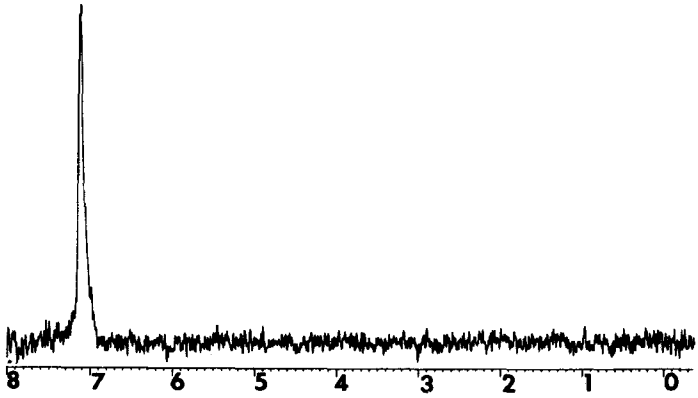


Figure 2. ^{3}H NMR (CD_3OD) of (±)-[phenoxy-^{3}H(N)] phenoxybenzamine·HCl (1). Chemical shift values are in parts per million downfield from internal $(CH_3)_4Si$.

to make the HCl salt of the radioligand). Product 1 was homogeneous on TLC (reverse phase - CH_3OH: CH_3CN (9: 1), Rf 0.6) and HPLC (μC_{18} - CH_3OH at 1 mL/min., retention time = 6 min.) and in these chromatographic systems it cochromatographed with authentic phenoxybenzamine·HCl (Tokyo Kasei cat # D158). Also, the UV (EtOH) spectrum of 1 was superimposable on that of authentic cold standard and a specific activity of 32.4 Ci/mmol was calculated for 1 by UV (EtOH) spectroscopy where ε_{272} = 1578 for authentic phenoxybenzamine·HCl. A ^{3}H NMR (CD_3OD) of 1 (Figure 2) afforded a single peak at δ 7.05 ppm indicative of exclusive aromatic ring radiolabeling.

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