THE PREPARATION AND CHARACTERIZATION OF [^{3}H] HISTAMINE.2HCl AT HIGH SPECIFIC ACTIVITY[1]

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SUMMARY

[^{3}H] Histamine.2HCl will continue to play a key role in establishing the neurotransmitter status of histamine.2HCl. To more consistently achieve high specific activity in the preparation of the radioligand and incorporate as much tritium into the stable side chain positions as possible, we explored the use of alternative tritiation precursors. Imidazole 4-acetonitrile (5) and 2,5-diiodoimidazole-4-acetonitrile (6) were catalytically reduced with tritium gas and the radiolabeling specificity of the resulting [^{3}H] histamine.2HCl produced was confirmed by ^{3}H NMR.

Key Words: [^{3}H] Histamine, Tritium Labeling, ^{3}H NMR, Catalytic Reduction

INTRODUCTION

The physiological actions of histamine.2HCl (1) were described as early as 1910,[2] and histaminergic is the term applied to a certain population of receptors (H_1 and H_2) that regulate body temperature, gastric secretion and certain smooth muscle contraction. Although mounting neurochemical evidence supports the role of 1 as a neurotransmitter in mammalian brain,[3], its status as such is still putative. To assist in firmly establishing the neurotransmitter role of 1, [^{3}H] histamine.2HCl will continue to be utilized in autoradiography, receptor binding assay[4,5] and electrophysiological studies.[6] [2-^{3}H] Histamine.2HCl (2) at low specific activity has been prepared by general exchange tritiation of 1,[7] and [2,5-^{3}H] histamine.2HCl (3) at high (25-50 Ci/mmol) specific activity has been prepared by the catalytic tritiation of 2,5-diiodohistamine (4).[8] To more consistently achieve high specific activity

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in the preparation of [^{3}H] histamine.2HCl and incorporate as much tritium into the stable side chain positions as possible, we explored the use of nitriles 5 and 6 as alternative precursors.

DISCUSSION

Nitrile 5 is commercially available. Although 1 can easily be diiodinated to 4,[9] and histidine·HCl (7) can be diiodinated to 2,5-diiodohistidine·HCl (8)[10], nitrile 5 could not be directly diiodinated to 2,5-diiodoimidazole-4-acetonitrile (6). A recent report[11] illustrated the difficulty and confusion that can attend the iodination of imidazoles. Exploiting the established procedure to prepare 5 from 7,[12] we found that novel compound 6 could be prepared in 31% yield by the treatment of 8 with sodium hypochlorite.

Nitrile 5 was catalytically reduced with tritium and PdO in acetic anhydride followed by hydrolysis to yield [ring-2, ethanamine-^{3}H] histamine.2HCl (9) at 44 Ci/mmol in 3.3% radiochemical yield. A ^{3}H NMR (CD_3OD, DCl - Figure 1) of 9 revealed that 23% of the radiolabel was incorporated in and retained by the 2-position with the remainder residing in the side chain. Monitoring the ^{3}H NMR of 9 in acidic solution at 4°C for several weeks showed that essentially no exchange of the 2-^{3}H occurred. This general exchange of tritium into the 2-position of 9 is reminiscent of the result observed when 1 is exposed to tritium gas in the presence of 5% Rh/Al_2O_3 affording 2 as shown by ^{3}H NMR.[7]

Hydrogenation of 6 with PdO in acetic anhydride did not yield any 1. To facilitate both the dehalogenation and clean nitrile reduction of 6, more basic conditions proved necessary. Exposure of 6 to tritium gas in EtOH:NH_4OH using both 10% Pd/C and 5% Rh/Al_2O_3 afforded [ring-5, ethanamine-^{3}H] histamine.2HCl (10) at 35 Ci/mmol in 17.3% radiochemical yield. A ^{3}H NMR (CD_3OD, DCl - Figure 2) of 10 revealed that 42% of the radiolabel was incorporated in and retained by the 5-position with the remainder in the side chain. When compound 6 was stirred with 10% Pd/C and 5% Rh/Al_2O_3 in EtOH:NH_4OH for 24 h it was recovered unchanged. Therefore, the absence of tritium in the 2-position of compound 10 occurred as a result of exchange with the solvent after tritiation. To better understand the factors governing the rate of exchange at the 2-position of 10, ^{2}H NMR studies were conducted. [2,5-^{2}H] Histamine·2HCl was prepared by the

catalytic deuteration of 4 and treated with EtOH:NH_4OH with and without 10% Pd/C and 5% Rh/Al_2O_3. Also, 1 was treated with EtOD:ND_4OD with and without 10% Pd/C and 5% Rh/Al_2O_3. In these parallel experiments it was observed by ^2H NMR that both catalyst and base were necessary for a facile exchange of the 2-position.

Both compounds 9 and 10 were purified by ascending paper chromatography and were found to be 98% radiochemically pure by TLC and HPLC, and also cochromatographed with authentic 1. The specific activity of 9 and 10 was determined by fluorescamine assay. Nitriles 5 and 6 have been shown to serve as useful precursors to [^3H] histamine·2HCl at high specific activity, incorporating a majority of the radiolabel in the stable side chain position.

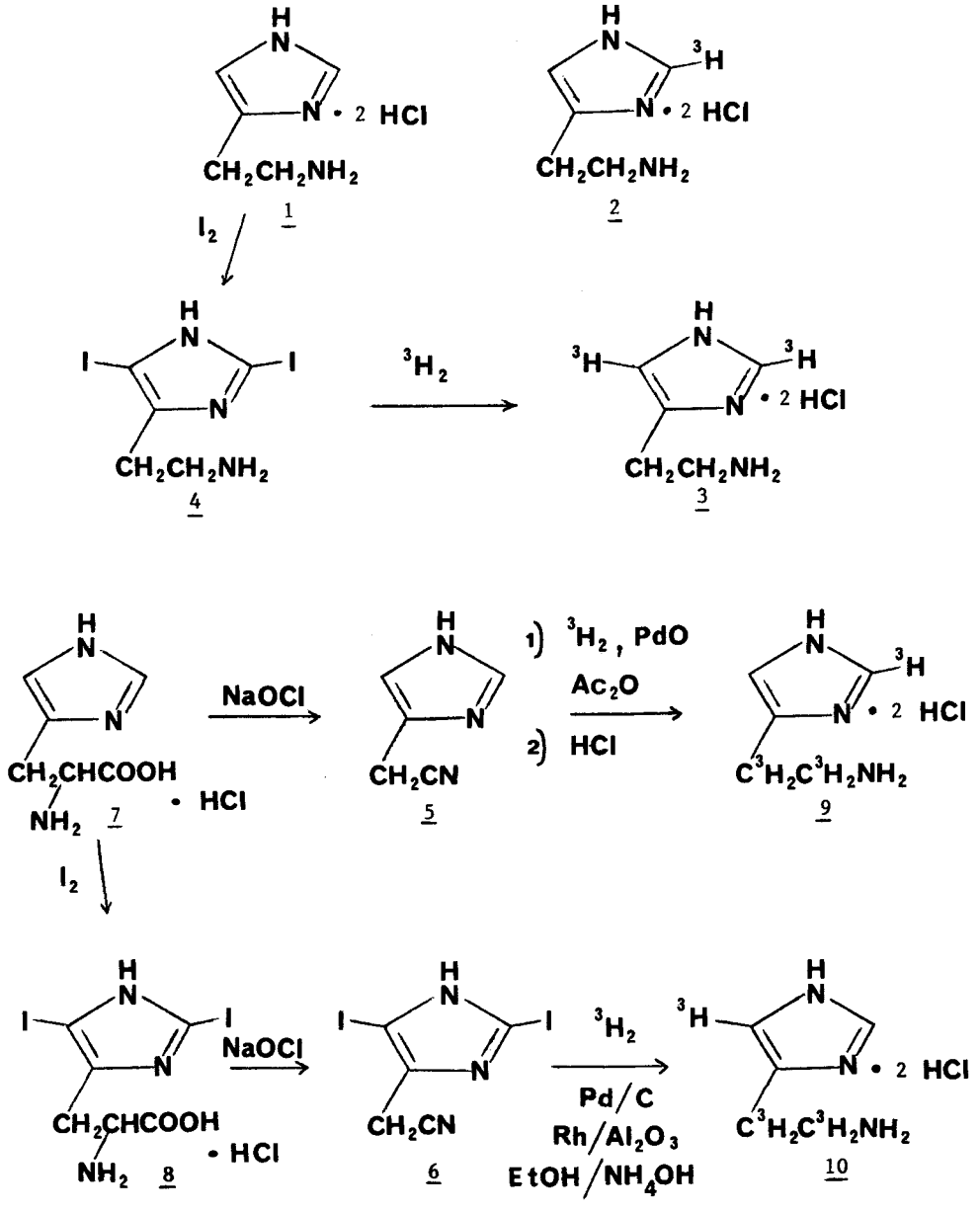
EXPERIMENTAL PROCEDURES

General Methods: Evaporations were carried out on a Buchi rotary evaporator. Analytical TLC was performed either on Analtech 5 x 15 cm silica gel or avicel GF coated glass plates. Common solvent combinations were S_1 ($CHCl_3$:CH_3OH (9:1)); S_2 (PhH:acetone (9:1)); S_3 (PhH:$CHCl_3$:EtOH (2:4:1)); S_4 (EtOH:NH_4OH (4:1)); S_5 (n-BuOH:HOAc:H_2O (25:4:10)). Autoradiography was performed at 0°C after spraying TLC plates with PPO (DuPont, NEN Research Products) and exposure to Eastman Kodak SB-5 film. The plates were also scanned for radioactivity by using a Packard 7201 scanner. Analytical HPLC determinations were run on a Waters instrument using a μ C_{18} column. Common solvent combinations were S_6 (0.01N KH_2PO_4 (pH = 2.5):CH_3OH (70:30)); S_7 (Pic B7:CH_3OH (70:30)). Peak detection was performed simultaneously by a liquid scintillation flow monitor and UV (254 nm) detection. The IR spectrum was measured on a Perkin-Elmer Model 700 spectrophotometer. The proton and triton magnetic resonance spectra were obtained on a Bruker WP 200 mHz NMR spectrometer. Chemical shift values are expressed in parts per million downfield form internal TMS or TSP. Fluorescence measurements were determined using a Turner Model 430 fluorimeter. The UV spectrum was measured on a Beckman model 25 spectrophotometer and the high resolution mass spectrum was performed by Shrader Analytical Laboratories (Detroit, MI).

2,5-Diiodoimidazole-4-acetonitrile (6). To a suspension of L-2,5-diiodohistidine. HCl[10] (8, 900 mg, 2.03 mmol) in 40 mL of H_2O at 0°C was added with vigorous stirring 6.30 mL of a 5.25% sodium hypochlorite solution (Chlorox) dropwise over the course of several min. Gas (CO_2) evolution was noted immediately. The reaction was allowed to warm to room temperature and stir overnight. After this time, the dark acidic solution was basified to pH 9 with excess Na_2CO_3 and cautiously evaporated to dryness (foaming). The residue was slurried with twenty 20 mL portions of EtOAc. The combined EtOAc washings were filtered and evaporated to yield 550 mg of a residue. The residue was preparatively chromatographed on six 1000 μm silica gel GF plates developed with S_1. The main band (R_f 0.7) was visualized by UV, scraped and eluted with EtOAc. Solvent evaporation yielded 225 mg (31%) of 6 as a white solid, m.p. 173-175°C, that was found to be homogeneous on TLC (Silica Gel - S_1, S_2, S_3) and HPLC (μ C_{18} - S_6) ^1H NMR (CD_3OD) δ3.77 ppm (s); ^{13}C NMR (CD_3OD) 117.46, 87.95, 79.49, 16.75 ppm; IR (KBr) 3080, 3000, 2900, 2820, 2650, 2270, 1555, 1475, 1400, 1050, 980 cm^{-1}; UV (EtOH) ε230 (10,840).

High resolution mass spectrum calcd for $C_5H_3N_3I_2$ (M^+):358.8421. Found 358.8416.

[Ring-2, Ethanamine-^3H] Histamine·2HCl (9). A solution of imidazole-4-acetonitrile (5, 11 mg, 0.1 mmol) in 3 mL of acetic anhydride with 25 mg of PdO was reduced with 70 Ci of tritium gas for 16 h at 24°C. Following catalyst filtration and removal of labile tritium, the residue was dissolved in 5 mL of 2N HCl and heated at 100°C for 16 h. Following solvent evaporation and removal of labile tritium with CH_3OH, the residue was dissolved in 10 mL CH_3OH:H_2O (1:1). The crude product (4015 mCi) was purified twice by ascending paper chromatography; first on three Whatman 3 mm sheets followed by purification on two Whatman 1 mm sheets developed each time overnight with i-PrOH:Conc·HCl:H_2O (26:8:6). To facilitate product location, cold histamine·2 HCl (1) was allowed to migrate at the side of each paper. After each overnight development, the papers were dried, autoradiogrammed, and the main radioactive band lining up with visualized (ninhydrin) cold standard histamine ·2HCl (R_f 0.5) was cut out and eluted with EtOH:0.01 N HCl (1:1) affording 146 mCi(3.3%


H
N
N • 2 HCl
CH2CH2NH2
1
3H
2
I2
4
3H2
3
NaOCl
7
• HCl
5
1) 3H2, PdO
Ac2O
2) HCl
9
C3H2C3H2NH2
8
6
Pd/C
Rh/Al2O3
EtOH/NH4OH
10

Scheme 1

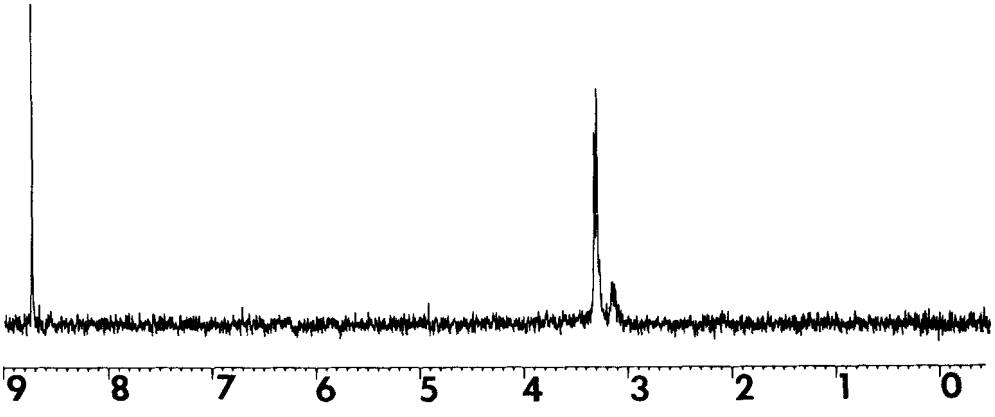


Figure 1. ^{3}H NMR (CD_3OD, DCl) of [ring-2,ethanamine-^{3}H] histamine· 2 HCl (9). Chemical shift values are in parts per million from internal TSP.

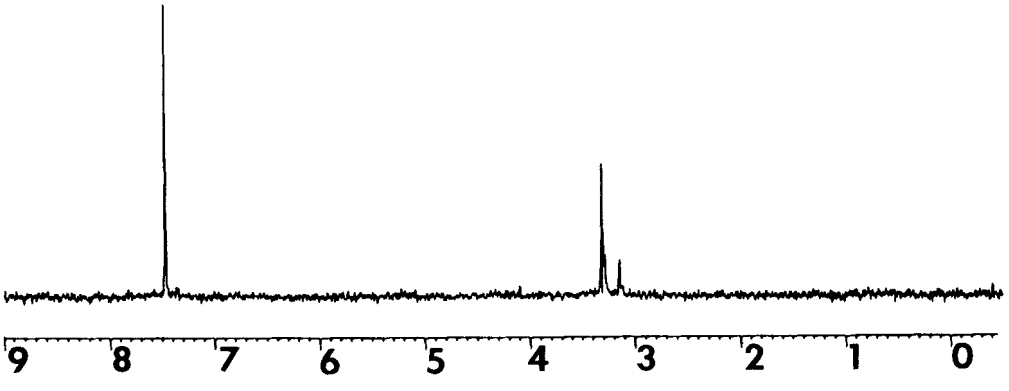


Figure 2. ^{3}H NMR (CD_3OD, DCl) of [ring-5,ethanamine-^{3}H] histamine·2 HCl (10). Chemical shift values are in parts per million from internal TSP.

radiochemical yield based on 5) of 9. Ligand 9 was found to be 98% radiochemically pure by TLC (silica gel - S_4, avicel - S_5) and HPLC (μ C_{18} - S_7) and in these systems 9 cochromatographed with authentic 1. A specific activity (fluorescamine) of 44 Ci/mmol was determined for 9. A ^3H NMR (CD_3OD,DCl) of 9 (Figure 1) afforded a singlet at δ 8.72 ppm and a multiplet at δ 3.40 - 3.00 ppm.

[Ring-5, Ethanamine-^3H] Histamine·2HCl (10). A solution of 2,5-diiodoimidazole-4-acetonitrile (6, 32 mg, 0.09 mmol) in 6 mL of EtOH:NH_4OH (2:1) with 20 mg each of 10% Pd/C and 5% Rh/Al_2O_3 catalyst was reduced with 80 Ci of tritium gas for 16 h at 24°C. Following catalyst filtration and removal of labile tritium, the residue was dissolved in 10 mL CH_3OH:H_2O (1:1). The crude product (1548 mCi) was purified by ascending paper chromatography on two Whatman 3 mm sheets developed overnight with i-PrOH:conc. HCl:H_2O (26:8:6). To facilitate product location, cold histamine·2HCl (1) was allowed to migrate at the side of each paper. After overnight development the papers were dried autoradiogrammed and the main radioactive band lining up with visualized (ninhydrin) cold standard histamine·2HCl (R_f 0.5) was cut out and eluted with EtOH:0.01 N HCl (1:1) affording 540 mCi (17.3% radiochemical yield based on 6) of 10. Ligand 10 was found to be 98% radiochemically pure by TLC (silica gel - S_4, avicel - S_5) and HPLC (μ C_{18} - S_7) and in these systems 10 cochromatographed with authentic 1. A specific activity (fluorescamine) of 35 Ci/mmol was determined for 10. A ^3H NMR (CD_3OD, DCl) of 10 (Figure 2) afforded a singlet at δ 7.47 ppm and a multiplet at δ 3.35 - 3.10 ppm.

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