THE PREPARATION OF DEUTERIUM- AND TRITIUM-LABELED PIRBUTEROL HYDROCHLORIDE

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SUMMARY

A mild exchange labeling procedure utilizing O-deuterated or O-tritiated t-butanol with sodium catalysis afforded isotope exchange in the α-hydrogen position of the amide N-t-butyl-2-(5-benzyloxy-6-hydroxymethyl-2-pyridyl)-2-hydroxy-acetamide, thereby extending the utility of this approach with ketones to poor enolizers that are base labile. Complete label retention accompanied the subsequent steps of reduction and debenzylation to 2-hydroxymethyl-3-hydroxy-6(1-hydroxy-2-t-butylaminoethyl)-pyridine dihydrochloride (pirbuterol hydrochloride)-^{2}H or -^{3}H, in 15-18% overall yield at the 4 mmole level. Deuterium incorporation was estimated to be 39%; a specific activity of 206 μc/mg was achieved by radiolabeling. A radiochemical and chemical purity of >99.5% was established. Tritium label stability was demonstrated in aqueous media at acidic, neutral and basic pH.

Key Words: Pirbuterol, Deuterium, Tritium, Catalytic Exchange, 2-Hydroxyacetamide

INTRODUCTION

Pirbuterol hydrochloride is a potent sympathomimetic bronchodilator in animal models(1) and is presently under clinical investigation for effectiveness in patients with chronic bronchospastic disease. To facilitate an understanding of the disposition of pirbuterol in animals during preclinical studies, it became of interest to examine means by which this agent could be radiolabeled. In view of the lengthy synthetic schemes required for Carbon-14 incorporation, direct tritium exchange labeling procedures with the parent compound were considered. As a guide to the success of this approach, trial experiments were conducted by deuterium exchange with pirbuterol and several model compounds.

A number of base-catalyzed systems which probed the exchangeability of ring hydrogen with deuterium oxide under conditions appropriate for ortho-labeling of phenolic hydroxyl groups were unsuccessful (Table I), attributable to the

Table I. Deuterium exchange reactions with pirbuterol and model compounds (0.05-1 mmole)

Compound	Conditions	Stability/Recovery[a]	Exchange[b]
1. Base catalysis			
Pirbuterol	D_2O/DMF (autocatalysis), 90°, 65 hr.	good	none
Pirbuterol	0.5 eq. TEA/D_2O, 90°, 40 hr.	good	none
Pirbuterol	0.5 eq. KOtBu/D_2O, 90°, 3 hr.	good	none
Pirbuterol	1 eq. TEA/D_2O (pH 9.6), 90°, 3 hr.	good	none
Pirbuterol	KOH/D_2O, pH 12, 90°	$t_{1/2}$, 6 hr.	--
p-Cresol(2)	0.5 eq. TEA/D_2O, (pH 10.6), 90°, 3 hr.	--	47% (d_2)
3-Hydroxypyridine	1 eq. TEA/D_2O (pH 10), 90°, 3 hr.	--	none
2,6-bis-(hydroxymethyl)-3-Hydroxypyridine	1 eq. TEA/D_2O (pH 10), 90°, 3 hr.	--	none
2,6-bis-(hydroxymethyl)-3-Hydroxypyridine[c]	3 N $NaOCH_3/CH_3OD$, 75°, 21 hr.	complete degradation	--
Pirbuterol	3 N $NaOCH_3/CH_3OD$, 75°, 21 hr.	complete degradation	--
3-Hydroxypyridine	0.5 N $NaOCH_3/CH_3OD$, 75°, 16 hr.	good	none
Toluene(4)	1.5 N KOtBu/$(CD_3)_2SO$, 65°, 18 hr.	good	100% ($-CH_2D$)
Benzylated pirbuterol, 3	1.5 N KOtBu/$(CD_3)_2SO$, R.T.,2→24 hr.	good/63→43%	none
Pirbuterol	1.5 N KOtBu/$(CD_3)_2SO$, R.T., 19 hr.	good/23%	none

Table I. (continued)

Compound	Conditions	Stability/Recovery[a]	Exchange[b]
Pirbuterol	t-BuOD/Na, R.T., 65 hr.	good	none
2. Acid catalysis			
3-Hydroxypyridine	4N DCl, 90°, 2 hr.	good	none
3-Hydroxypyridine	pH 2 DCl, 90°, 2 hr.	good	none
Pirbuterol	D_2SO_4/CH_3OD, R.T., 1 hr.	good/55%	none
Pirbuterol	D_2O + 30% SO_3/H_2SO_4, R.T., 1 hr.	variable	none
3. Transition-metal catalytic exchange[d]			
2,6-bis-(hydroxymethyl)-3-Hydroxypyridine	Pt/D_2O, 75°, 3.5 hr.	minor degradation	none
3-Hydroxypyridine	Pt/70% CD_3COOD, 100°, 24 hr.	minor degradation	none

[a] Stability/Recovery determined by TLC and GLC assay of isolated product (by evaporation or by the acetone-K_2CO_3 extraction method).
[b] Percent deuterium incorporation, determined by mass spectrometric analysis of isolated product washed free of labile deuterium.
[c] Conditions based on those employed for the preparation of 3-chloro-pyridine-4-^2H (3).
[d] Conditions based on those employed for the preparation of tritiated pyridoxine·HCl (5).

presence of a substituted pyridine which would be highly inactivated to exchange of the available aromatic protons. The more rigorous conditions of alkoxide exposure in labeled anhydrous solvents, necessary for the exchange of ring hydrogen in certain model substituted pyridines, and of exocyclic hydrogen in toluene, resulted in the extensive degradation of pirbuterol with no evidence of deuterium incorporation (Table I). A further examination of exchange reactions by acid and transition-metal catalysis also afforded no labeled product.

With this information, attention was directed to an amide intermediate 1 of the pirbuterol synthesis which offered a weakly acidic α-hydrogen for potential exchange. The reluctance of amides to submit to deuterium exchange is sometimes overcome with O-deuterated alcohols in the presence of alkoxide(6), conditions which led to the decomposition of amide 1. However, the α-exchange of 1, conducted as with ketones in the presence of deuterioalcohols and catalytic amounts of sodium(7,8), conveniently afforded appreciable incorporation of one deuterium atom in good yield. Reaction conditions were optimized with the use of t-butanol-O-^{2}H. Subsequent amide reduction and debenzylation to pirbuterol-^{2}H hydrochloride proceeded with full label retention. The method was readily adaptable to tritium exchange with t-butanol-O-^{3}H, ultimately providing pirbuterol-^{3}H hydrochloride with a high specific activity in an overall yield of 18%. This radiolabeling procedure presented both cost and time advantages relative to the option of preparing tritium-labeled pirbuterol by amide reduction with tritiated diborane; furthermore, the position of label in the latter product would be less desirable from the standpoint of possible metabolic vulnerability(9-11).

The following section describes the procedures by which the deuterium- and tritium-labeled preparations of pirbuterol hydrochloride were obtained (Fig. 1) and analyzed.

EXPERIMENTAL

Analytical Methods

Thin layer chromatography (TLC) was conducted with precoated silica gel glass plates (E. Merck) and solvent systems a, b and c:

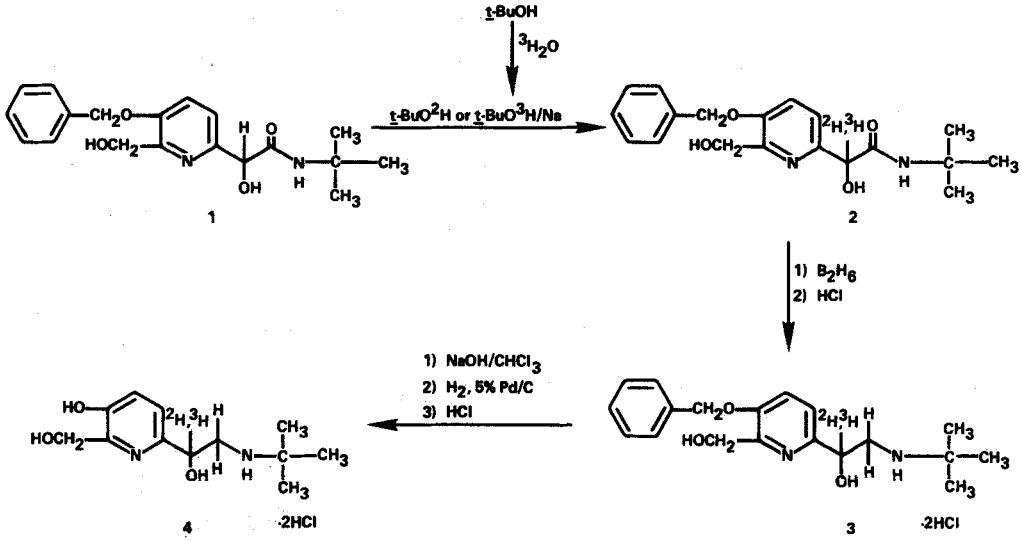


Fig. 1. Synthesis of deuterium- and tritium-labeled 2-hydroxymethyl-3-hydroxy-6-(1-hydroxy-2-t-butylaminoethyl) pyridine dihydrochloride (pirbuterol hydrochloride, 4)

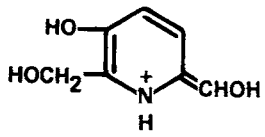
	R_f		
	2	3	4
a. ethyl acetate-diethylamine (95:5)	0.44	0.21	0
b. benzene-ethyl acetate-glacial acetic acid (100:100:15)	-	-	0.05
c. methyl ethyl ketone-glacial acetic acid-water (6:1:1)	0.81	0.47	0.30

Radiochemical purity was estimated with the aid of a Varian Aerograph Model 6000-2 radiochromatogram scanner. Radiolabeled zones were visualized by autoradiography with Eastman Blue Brand X-ray film. Ultraviolet (UV) absorption data from 0.1N methanolic HCl solutions (296 nm) assisted purity determination. Gas liquid chromatography (GLC) analysis of purity and tritium label stability of product was performed with a Microtech Model MT-200 gas chromatograph equipped with a

2 m 3% OV-225 glass column (4 mm i.d., Gas-Chrom Q, 60-80 mesh) and a flame ionization detector. Samples were injected following the preparation of trimethylsilyl (TMS) ether derivatives with 50% Regisil (BSTFA + 1% TMCS) in acetone. At a column temperature of 180°, 4-$(TMS)_3$ exhibited a retention time of 2.1 min. Solid probe mass spectrometry (MS) was conducted with an LKB 9000 mass spectrometer at ionization potentials of 70 and 12-13 eV, to assist product identification and deuterium incorporation analysis. Deuterium incorporation with pirbuterol, which exhibits a very weak molecular ion signal, was determined by reference to the decline in base peak intensity at m/e 155 and the increased ion abundance at m/e 156 (Table II).

Table II. Relative fragment ion intensities of pirbuterol and pirbuterol-^2H.

m/e	Relative ion intensity	
	Pirbuterol	Pirbuterol-^2H
155	100	100 (d_0)
156	8.3	73.3 (d_1)
157	1.5	7.1 (d_2)
158	0.7	0.9 (d_3)



m/e 155

Proton NMR spectra of 4-^2H were obtained at 60 MHz in deuterium oxide with sodium DSS internal reference. Radioactivity determinations were obtained by conventional liquid scintillation spectrometric methods.

The New England Nuclear Corp. prepared the tritium labeled t-BuOH and conducted the exchange reaction with 1 according to a protocol developed for the deuterium exchange.

Preparation of Deuterium-Labeled Pirbuterol Hydrochloride (4-^2H)

1. Hydrogen Deuterium Exchange. N-t-butyl-2-(5-benzyloxy-6-hydroxymethyl-2-pyridyl)-2-hydroxyacetamide-$^2H_1$ (2-^2H).

In a stoppered 100 ml round bottom flask and in an atmosphere of nitrogen, 1.378 g (4 mmole) of 1 was dispersed in 16 ml (166 mmole) of freshly distilled t-butanol-^2H (Aldrich, 98+% $(CH_3)_3COD$). To the dispersion was added

37 mg of sodium metal in a subdivided state, and the system was magnetically stirred for a period of 20 hours at room temperature under nitrogen. To the final solution chilled to 0° with an ice bath was added 4 ml of water, and the contents were lyophilized. The dried product was freed of labile deuterium in vacuo, using methanol as solvent, and extracted from 2 ml of water (pH 10) with 10 ml of chloroform. After drying with Na_2SO_4, solvent removal in vacuo afforded 1.491 g (108%) free base oil 2-^{2}H. Authenticity was confirmed by comparative TLC (>90%, single component), GLC and MS (39% d_1 incorporation) analysis with 2 reference standard.

2. Reduction to 2-hydroxymethyl-3-benzyloxy-6-(1-hydroxy-2-t-butylaminoethyl) pyridine-$^2H_1$ dihydrochloride (3-^{2}H).

Free base oil 2-^{2}H (4 mmole) in 24 ml of anhydrous tetrahydrofuran was added from a dropping funnel to a 100 ml RB flask containing 12 ml of a 1 M solution of diborane in tetrahydrofuran (Aldrich) over a 1-2 hour period, controlled by adjusting the amount of hydrogen evolution while maintaining a temperature of 0-5°. The temperature of the reaction medium was then slowly raised (30 min) to room temperature and heated at reflux (65°) for an additional 1.5 hours. TLC analysis at this point indicated that amide reduction to 3-^{2}H was essentially complete. The contents were cooled to room temperature and treated with 2.5 ml of ethanolic HCl (4.5 N) and 50 ml of diethyl ether to yield, after overnight chilling, filtration and vacuum drying, 0.894 g (55%) crystalline 3-^{2}H. The product was identical with an authentic sample of unlabeled 3 with respect to TLC (>90%, single component) m.p. (185-187°, dec.), GLC and MS (39% d_1 incorporation) data.

3. Hydrogenation to 2-hydroxymethyl-3-hydroxy-6-(1-hydroxy-2-t-butylaminoethyl) pyridine-$^2H_1$ dihydrochloride (pirbuterol-^{2}H hydrochloride; 4-^{2}H).

Three-^{2}H was reconstituted in 2 ml of water (pH 10.5) and extracted with 4 x 10 ml of chloroform. The combined extracts were dried with Na_2SO_4 and reduced to dryness in vacuo to afford 0.600 g (1.82 mmole) 3-^{2}H free base. The compound was hydrogenated in a Parr pressure bottle in the presence

of 9 ml of 96% methanol and 0.34 g of 5% palladium/carbon, 50% H_2O catalyst (Englehard) at 50 psi for 2 hours. The product was sintered glass (UF)-filtered, reduced to an oil in vacuo, dissolved in 10 ml of water and lyophilized. To the resulting residue in 1-2 ml of ethanol was slowly added 0.65 ml of 8N ethanolic HCl and 11 ml of isopropyl ether at 0-5°C to induce slow crystallization of crude hydrochloride. Chilling, filtration and vacuum drying gave 0.246 g 4-^{2}H, confirmed by TLC, GLC and MS (39% d_1 incorporation) analysis. Recrystallization from methanol-isopropyl ether (6:1), after slurrying in the presence of 50 mg Darco KB, provided 0.192 g (28%; 15% overall) 4-^{2}H, m.p. 166-167° (ref. 176-178°). Purification was achieved by free base regeneration from acetone and triethylamine (molar eq.), and dihydrochloride crystallization from the resulting filtrate with methanol and 5.5N ethanolic HCl to afford 0.100 g of 4-^{2}H, m.p. 177-178° (ref. 176-178°). Purity was established by TLC (single component), UV and MS (39% d_1 incorporation) analysis. NMR evidence supported the site of expected deuterium exchange: integrated area under the quartet signal assigned to the side chain β-hydrogen (C-1' methine proton, δ=5.55, J=4 cps) averaged 65% of that measured for the unlabeled material.

Preparation of Tritium-Labeled Pirbuterol Hydrochloride (4-^{3}H)

1. Hydrogen Tritium Exchange. N-t-butyl-2-(5-benzyloxy-6-hydroxymethyl-2-pyridyl)-2-hydroxyacetamide-$^3H_1$ (2-^{3}H).

Amide 1 (4 mmole) was subjected to tritium exchange with O-tritiated t-butanol under conditions as described above for the deuterium labeling. The tritiated alcohol was prepared by distilling 30 curies of ^3H_2O into 3 ml of freshly distilled t-butanol. Following a 30 min. stirring period to effect exchange, molecular sieve was added to absorb water. The t-butanol-^{3}H was distilled into an exchange reaction flask containing 1 in 13 ml of freshly distilled t-butanol and 37 mg sodium. Following a 16-hour exchange period, labile tritium was removed in vacuo, using methanol as wash solvent, and dried in vacuo. Extraction and recovery of product from 2 ml of water (pH 10) with

chloroform in the normal manner provided 1.184 g (86%), 133 μCi/mg, free base oil 2-^{3}H, TLC (2 minor impurities) and MS data confirmed the presence of authentic 2-^{3}H.

2. Reduction to 2-hydroxymethyl-3-benzyloxy-6-(1-hydroxy-2-t-butylaminoethyl) pyridine-$^{3}H_{1}$ dihydrochloride (3-^{3}H).

Free base oil 3-^{3}H (3.44 mmole) was reduced in tetrahydrofuran with diborane under conditions as described above for the deuterated reduction procedure, with the appropriate molar equivalent scale adjustments. Crystalline dihydrochloride 3-^{3}H, 0.883 g (66%) was obtained which exhibited analytical properties comparable to those of authentic unlabeled 3.

3. Hydrogenation to 2-hydroxymethyl-3-hydroxy-6-(1-hydroxy-2-t-butylaminoethyl) pyridine-$^{3}H_{1}$ dihydrochloride (pirbuterol-^{3}H hydrochloride; 4-^{3}H).

Three-^{3}H (2.2 mmole) was converted to the free base and hydrogenated under conditions as described for the reaction with the deuterated intermediate. Crude dihydrochloride 4-^{3}H, 0.311 g, separated from the ethanol-isopropyl ether solution and was recrystallized with methanol-isopropyl alcohol after Darco KB decoloration to give 0.222 g (33%; 18% overall) 4-^{3}H, m.p. 168-173° (ref. 176-178°), 143 μCi/mg. Two successive purifications by the acetone-triethylamine base regeneration method above afforded 0.106 g of 4-^{3}H, m.p. 177-179° (ref. 176-178°), 206 μCi/mg. TLC autoradiography in 3 systems revealed >99.5% radiochemical purity, with no evidence of unlabeled impurities responsive to 254 nm UV light exposure. Anal. for analytical grade reference pirbuterol hydrochloride: C, 45.0; H, 7.02; N, 8.71; Found: C, 44.98; H, 6.94; N, 8.50. The product was indistinguishable from authentic unlabeled pirbuterol hydrochloride on the basis of UV, IR (KBr) and MS analysis. Purity estimations were 105.5% and 100.0% by GLC and UV determinations, respectively.

A preparation of lower specific activity product was obtained for general metabolism studies by dilution of a portion of 4-^{3}H with 5 parts of unlabeled standard 4, as the free base in acetone, and formation in the usual manner of recrystallized hydrochloride (m.p. 178-179°), estimated by UV, TLC and

GLC analysis to be 100% radiochemically and chemically pure. This dilution step also served to eliminate degradation of the compound by self irradiation on storage (4°) in the solid state, which was found to proceed at a rate averaging 0.3%/month for the undiluted material.

Tritium Label Stability

The chemical stability of the tritium label of pirbuterol-^{3}H hydrochloride was determined in aqueous media at acidic, neutral and basic pH, accordingly: 4-^{3}H diluted with unlabeled reference pirbuterol hydrochloride was adjusted to 10 µg/ml concentrations (175,000 dpm/ml) in 20 ml of 1N HCl (25°), 0.1N NaOH (25°) or 0.1M phosphate buffer, pH 7.4 (37°). Two-ml aliquots were removed from the buffer solutions at 0, 2, 6, 24 and 48 hours and from the remaining aqueous

Table III. Tritium label stability studies with pirbuterol-^{3}H in aqueous solution.

Conditions	Time interval	Percent radioactivity recovery: Lyophilate H_2O	Lyophilate residue	Acetone extraction[a]	Percent pirbuterol content (GL
pH 7.4 buffer,	0 hr.	<0.1	104.8	91.1	100 ref.
37°	2 hr.	<0.1	102.1	94.4	100.0
	6 hr.	2.6	90.0	96.1	98.5
	24 hr.	0.6	96.7	100.3	92.3
	48 hr.	0.3	92.5	101.0	107.7
0.1 N NaOH, 25°	0 hr.	0.2	97.6	93.4	100 ref.
	48 hr.	<0.1	112.4	98.0	96.8
1 N HCl, 25°	0 hr.	0.2	97.6	96.3	100 ref.
	48 hr.	<0.1	104.3	96.5	101.1

[a]TLC examination (system c) of lyophilate residues and acetone extracts supported the absence of detectable pirbuterol degradation products.

systems at 0 and 48 hours only. One ml of each aliquot was lyophilized; the water condensate and the resulting residue were radioassayed. The remaining 1 ml was shaken with 5 ml of acetone and 1.7 g of $K_2CO_3 \cdot 1.5\ H_2O$, and centrifuged. The acetone layer was removed, evaporated to dryness and reconstituted with 0.1 ml acetone for GLC analysis of intact pirbuterol content. All assays were performed in duplicate. The averaged results, summarized in Table III, indicate that little to none of the incorporated tritium is exchanged within the 48-hour test period over the pH range employed; negligible amounts of tritium water and no degradation products were detected by TLC and GLC analysis. More rigorous conditions of drug exposure at neutral and alkaline pH are known to result in the chemical decomposition of pirbuterol, and therefore these conditions were not explored with the tritium labeled compound. The high degree of radiochemical stability of pirbuterol-^3H, therefore, suggests this preparation will be suitable for drug disposition studies in animal models.

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