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SYNTHESIS OF ^{14}C-RADIOLABELED RACTOPAMINE HYDROCHLORIDE

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Summary

Ractopamine HCl was uniformly labeled with carbon-14 in one of two phenyl rings as a requirement for animal metabolism studies. The six-step synthesis was completed in a 14% yield. Product instability on silica gel complicated purification, but development of a chromatographic method afforded ractopamine·HCl-^{14}C with a radiochemical purity of 98.2%.

Key Words: Ractopamine hydrochloride, carbon-14, radiolabeled, phenethanolamine, repartitioning agent

Introduction

Ractopamine·HCl (EL-737) is a phenethanolamine repartitioning agent with beta-adrenergic agonist properties which promotes growth and carcass leanness when fed to swine (1-4). It is currently under development by Eli Lilly and Company for this purpose. A racemic mixture of ractopamine·HCl-^{14}C was prepared for animal studies incorporating the label into a metabolically stable portion of the molecule. A 50:50 mixture of diastereoisomers was required which is the ratio obtained in production.

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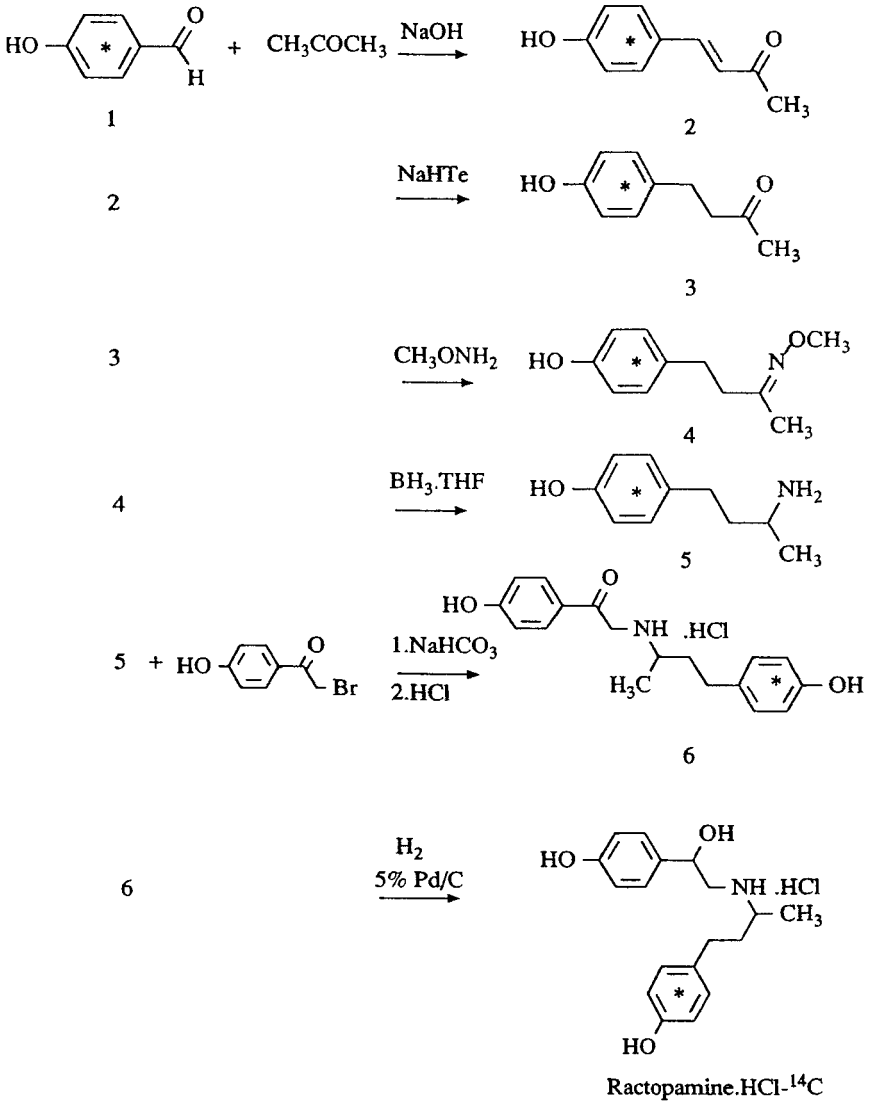
Results and Discussion

Historically both phenyl groups in the molecule have been radiolabeled separately (5). However, this paper will describe only the phenyl label outlined in Scheme I.

Aldol condensation (6,7) between 4-hydroxybenzaldehyde-UL-^{14}C 1 and acetone under basic conditions yielded the unsaturated ketone

SCHEME I



2. Selective reduction (8) of the unsaturated bond without concurrent carbonyl reduction was accomplished with sodium hydrogen telluride yielding the saturated ketone 3. Conversion of the carbonyl function to the O-methyloxime 4 was followed by reduction (9) with borane·THF complex to furnish compound 5. Alkylation with 4-hydroxyphenacyl bromide (10) in the presence of $NaHCO_3$, yielded ketoamine 6 plus some dialkylated material. Ketoamine 6 was converted to the hydrochloride salt and reduced catalytically while controlling the pH to afford crude ractopamine·HCl. Since ractopamine·HCl has been found to exhibit instability on silica gel, a chromatographic method was developed that allowed for its purification with minimal loss through decomposition. While this method involved a number of chromatographic separations, it provided ractopamine·HCl-^{14}C with a radiochemical purity of 98.2%.

Experimental

4-Hydroxybenzaldehyde-UL-^{14}C was acquired from Sigma Chemical Company. Radioactivity was determined using a Packard Tri-Carb Liquid Scintillation Counter, Model 300C. Thin-layer chromatography (TLC) was performed on 5 x 20 cm 0.25-mm precoated silica gel plates (Merck 60 F_{254}). Radioactive purity was determined by HPLC using a ConstaMetric III pump and a Model 7125 sample injector (Rheodyne Inc.). Two columns were used: a 5 µ, 10 x 250 mm Ultrasphere-Si normal phase (Beckman-Altex) and a 5 µ, 10 x 250 mm IBM Phenyl reverse phase (IBM Instruments, Inc). The mobile phases were CH_2Cl_2:CH_3OH:conc.NH_4OH, 75:25:2 (V/V/V) and CH_3CN/0.1M NH_4HCO_3 buffer; pH= 10 and 3.2 for the normal and reverse phase columns, respectively. In all instances, 1-ml fractions were collected on a fraction collector. The location of the radioactivity was determined by counting each fraction.

Quantitation was achieved by the addition of an internal standard. The isomeric ratio of the ^{14}C and unlabeled ractopamine·HCl was determined by HPLC with UV detection at 226 nm. The ractopamine·HCl-^{14}C was dissolved in 0.5M $NH_4H_2PO_4$:CH_3CN, 4:1, (V/V) to afford concentrations of approximately 50 µg/ml. A suitable aliquot was injected onto an IBM C18 column (4.5 x 250 mm, 5µ particles). The column was eluted with 0.5M $NH_4H_2PO_4$ buffer:CH_3CN:triethylamine, 88:11:1; two peaks were observed. From reference standards of RR and RS isomers, the elution order of the peaks was assigned RS, SR and RR, SS isomers, respectively. The ^1H NMR spectrum was measured on a Bruker WM-250 (MHz) spectrometer. The spectrum was recorded in DMSO-d_6 with Me_4Si as internal standard. The mass spectrum was measured on a VG Analytical Instruments ZAB-2SE mass spectrometer, cesium ion gun, and using "magic bullet" as the fast atom bombardment matrix.

4-(4-Hydroxyphenyl)-3-buten-2-one-Ph-UL-^{14}C (2)

A solution of 4-Hydroxybenzaldehyde-UL-^{14}C (10 mmol, 7.17 mCi/mmole, 73 mCi) dissolved in 15 ml of acetone and 10 ml distilled water was cooled to -5°C in an ice/alcohol bath. A 2-ml aliquot of 10N NaOH was added over 1-2 minutes while stirring under an atmosphere of nitrogen. The cooling bath was removed and the solution stirred at room temperature for 24 hours. Excess acetone was evaporated. The solution was cooled in an ice bath and acidified (pH=2) with conc. HCl (4 ml). The mixture was extracted with EtOAc (2 x 50 ml) and the extracts dried ($MgSO_4$). Evaporation of the filtrate yielded the crude product (2040 mg, weight>theory) as an orange-colored solid.

4-(4-Hydroxyphenyl)butan-2-one-Ph-UL-^{14}C (3)

Tellurium powder (1.56 g, 12 mmole) and $NaBH_4$ (1.06 g, 27.6 mmole) were suspended in 25 ml of absolute ethanol with stirring under an

atmosphere of nitrogen. The mixture was heated at reflux to initiate the exothermic reaction. and refluxing continued for 30 minutes after the exotherm subsided. The resultant purple-colored solution was allowed to cool to room temperature and a solution of 2 in 6 ml of C_2H_5OH added. The solution was stirred for 2 hours; tellurium precipitated. The mixture was transferred to a 250 ml beaker, acidified with 30 ml of 2N HCl, and stirred for 30 minutes while being exposed to the air. The mixture was filtered through a layer of Hyflo and the filter rinsed with C_2H_5OH. The filtrate was evaporated and the mixture extracted with EtOAc (2 x 50 ml). The EtOAc solution was dried ($MgSO_4$), filtered, and the filtrate concentrated in vacuo yielding the crude product as a reddish oil. Purification using low-pressure HPLC on silica gel (Woelm 04530, 250 g) eluting initially with CH_2Cl_2 and subsequently with CH_2Cl_2:Et_2O (95:5 and 90:10 V/V) yielded 1600 mg of 3 (97% yield) as a white crystalline solid.

4-(4-Hydroxyphenyl)butan-2-one-O-methyloxime-Ph-UL-^{14}C (4)

Powdered KOH (1609 mg, 28.73 mmol) was dissolved in absolute CH_3OH (25 ml) and the solution cooled in an ice bath under an atmosphere of nitrogen. A solution of methoxyamine.HCl (2440 mg, 29.22 mmol) in 15 ml of CH_3OH was added followed by a solution of 3 in 10 ml of CH_3OH. The mixture was heated at 45°C for 16 hours. The solvent was removed in vacuo and the residue treated with water (50 ml) and extracted with Et_2O (2 x 50 ml). The extracts were dried ($MgSO_4$) and concentrated in vacuo yielding 1880 mg (quant. yield) of 4 as a colorless oil.

2-Amino-4-(4-hydroxyphenyl)butane-Ph-UL-^{14}C (5)

Tracer 4 (1880 mg, 9.73 mmol) was dissolved in anhydrous THF (20 ml) with stirring under an atmosphere of nitrogen. The solution was cooled to -5°C with an ice/alcohol bath the BH_3·THF complex

(Aldrich, 1M, 26 ml) added over 20 minutes. The cooling bath was removed and the solution heated at reflux for 16 hours. The solution was cooled in an ice bath, and 20 ml of methanol was cautiously added. After foaming had ceased, the solution was evaporated. The residue was treated with 10% HCl (50 ml), and heated at reflux for 3 hours. The solution was cooled in an ice bath and its pH adjusted to 10.5 on a pH meter with 50% NaOH. The mixture was extracted with EtOAc (3 x 50 ml), the extracts dried ($MgSO_4$), filtered, and the filtrate evaporated to a constant weight yielding 1400 mg of 5 (87% yield) as a tan solid.

d,l-4-Hydroxy-a-[[[3-(4-hydroxyphenyl)-1-methylpropyl]aminol methyl]phenylketone·HCl-Ph-UL-^{14}C (6)

4-Hydroxyphenacylbromide was prepared in the following manner and reacted without further purification: 4-hydroxyacetophenone (Aldrich, 1153 mg, 8.47 mmol) was dissolved in 1,2-dimethoxy-ethane (DME, 25 ml) at room temp. 4-(Dimethylamino) pyridinium-bromide perbromide (Aldrich, 3074 mg, 8.47 mmol) was added in one portion and the mixture stirred at 25°C for 3 hours using a stream of nitrogen to expel the HBr. The mixture was poured into 50 ml of H_2O and extracted with EtOAc (2 x 50 ml). The extracts were washed with brine, dried ($MgSO_4$), and the filtrate concentrated to ca. 20 ml under reduced pressure. Tracer 5 was dissolved in EtOAc (15 ml) and H_2O (25 ml), $NaHCO_3$ (2.6 g, 30.5 mmol) added, and the mixture heated at 57°C with stirring. The solution of 4-hydroxyphenacylbromide (20 ml) was added over a 4 hour period. Upon completion of the addition, the mixture was stirred an additional 6 hours at 57°C and 24 hours at ambient temperature; the product precipitated. The mixture was cooled in an ice bath to 10-15°C, stirred 1 hour and filtered. The solid was washed with 50 ml of H_2O and 15 ml of EtOAc, and dissolved in 100 ml of CH_3OH. The solution was treated with 1N HCl (10 ml) and evaporated to dryness yielding 930 mg of 6 (33% yield).

d,l-4-Hydroxy-a-[[[3-(4-hydroxyphenyl)-1-methylpropyl]amino] methyl]benzenemethanol·HCl-Ph-UL-^{14}C (Ractopamine·HCl-^{14}C)

Tracer 6 was dissolved in 100 ml of CH_3OH. Water (5 ml) was added, and the pH of the solution adjusted to 6.4-6.5 by the addition of 1N NaOH. The solution was placed in a 500 ml Parr bottle and an aqueous slurry of 5% Pd/C (75 mg, 3 ml H_2O) added The reactor was pressurized with hydrogen at 50-60 psi, and shaken for 18 hours at 35°C. The mixture was filtered through a layer of Hyflo and the filtrate adjusted to a pH of 5.3-5.7 with 1N HCl. The filtrate was evaporated and the residue treated with absolute ethanol (3 x 100 ml), and evaporated repeatedly to azeotrope H_2O which afforded 963 mg of crude ractopamine·HCl-^{14}C as an oil. The crude product was dissolved in 18 ml of the solvent mixture: $CHCl_3$:C_2H_5OH:CH_3CN, 40:10:1 (V/V/V), and small amounts (3-ml aliquots, ~200 mg) were chromatographed (low-pressure HPLC on short columns of silica gel (Woelm 04530, 50 g, short residence time) saturated prior to the separation with the solvent mixture to minimize decomposition losses. As an added precaution, methanol was not substituted for ethanol in the solvent mixture, because it caused extensive decomposition of ractopamine·HCl-^{14}C on silica gel. The product was isolated in 15-ml fractions. Fractions 1-16 contained impure product whereas pure product was isolated in fractions 16-31. Seven such chromatographies were performed including those for the impure fractions. Evaporation of the solvent from the pure product yielded a viscous yellow oil. This oil was converted into a solid by repeated by trituration with n-hexane. The process afforded 480 mg (14% yield) of 98.2% radiochemically pure ractopamine·HCl-^{14}C with a specific activity of 5.81 mCi/mmol; ^{1}H NMR (DMSO-d_6): δ 9.43 (1H, S, 4'-HOAr); 9.174/9.186 (1H, RRSS/RSSR, 4" -HOAr); 8.44 (2H, NH^+HCl); 7.18 (2H, d, J_{ortho}=8.6 Hz, H2', H6'); 6.99 (2H, d, J_{ortho}=8.6 Hz, H3', H5'); 6.67 (2H, d, J_{ortho}=8.5 Hz,H3", H5"); 5.97 (1H, dd, HOC);

4.73 (1H, m, HCO); 3.15/2.99 (2H, m,NCH_2); 2.99 (1H, m, NCHMe); 2.50 (2H, m, CH_2Ar); 2.00/1.69 (2H, m, CH_2CH_2Ar); 1.26/1.22 (3H, d' s, J=6.2 Hz, CH_3C). FAB-MS m/z: 302.2 (M^+ -HCl+H, $C_{18}H_{24}NO_3$).

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