SYNTHESIS OF TRITIATED CLENBUTEROL

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SUMMARY

Tritiated clenbuterol was prepared starting from 4-aminoacetophenone (I) which was selectively brominated to 4-amino-3,5-dibromoacetophenone (II), then to 4-amino-α,3,5-tribromoacetophenone (III) and reacted with tert.butylamine to 4-amino-3,5-dibromo-α-tert.butylaminoacetophenone(IV). (IV) was dehalogenated and reduced with tritium gas to give 2-(tert.butylamino)-1-(4-amino-[3,5-^{3}H]-phenyl)-[1-^{3}H]-ethanol (V). This tritiated compound underwent selective aromatic chlorination to give the desired 2-(tert.butylamino)-1-(4-amino-3,5-dichlorophenyl)-[1-^{3}H]-ethanol, [ethanol-1-^{3}H]clenbuterol, with specific activity of 13.4 Ci/mmol (496 GBq/mmol).

Key words clenbuterol, tritium labelling, synthesis

INTRODUCTION

Clenbuterol is a substituted phenylethanolamine with β_2 sympathomimetic activity[1,2].

The synthesis of clenbuterol [2-(tert.butylamino)-1-(4-amino-3,5-dichlorophenyl)-1-ethanol] and of similar compounds has been

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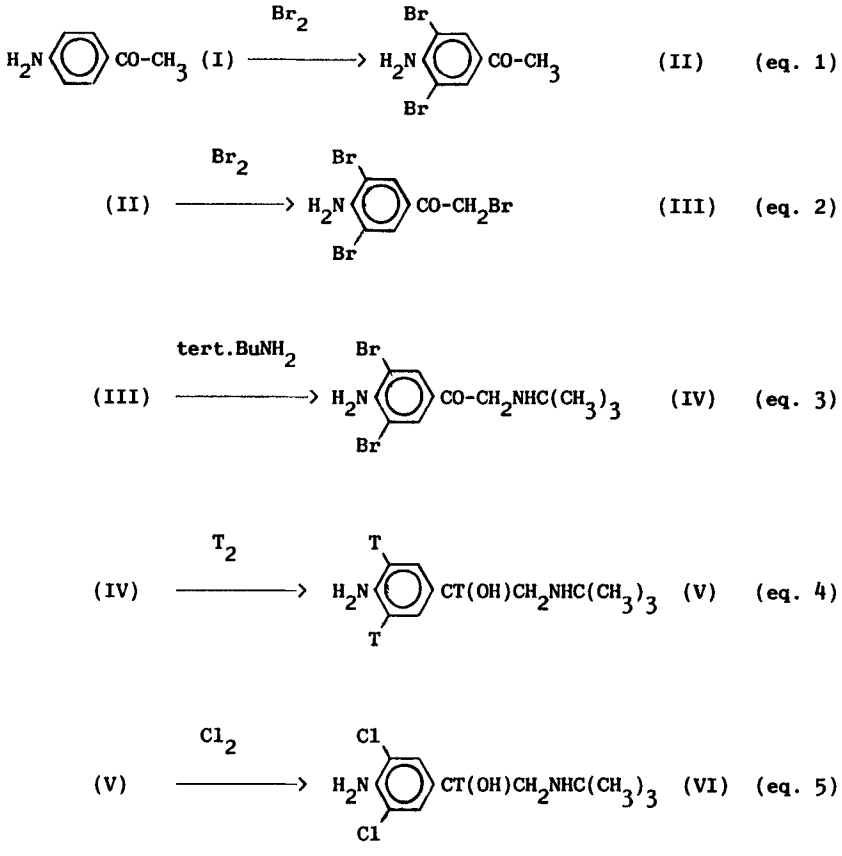
published[3], describing three different schemes all of which gave very good results. We wished to apply one of these schemes to a radioactive synthesis of tritiated clenbuterol and it seemed to us that the most suitable way would be to start with the keto derivative obtainable by oxidation of clenbuterol and reduce it thereafter with $NaBT_4$.

RESULTS AND DISCUSSION

The oxidation step from alcohol to ketone of similar compounds has already been mentioned in the patent literature[4]. However, all our attempts to oxidize clenbuterol, using various oxidizers known for their ability to convert secondary benzylic alcohols into ketones, resulted in unsatisfactory outcomes. For example, we tried: MnO_2[5,6,7], photochemical oxidation by dimethylsulfoxide[8], DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone)[9], and oxidation with CrO_3[4]. The oxidations were attempted under both ambient and elevated temperatures. In most of the cases, the oxidation did not occur at all. In some trials, splitting of the side-chain gave a benzaldehyde derivative, as observed from the NMR and the IR spectrum of the products mixture.

We therefore decided to prepare the keto derivative by a three-step synthesis, starting from p-aminoacetophenone (eq. 1-3). Attempts to brominate directly the methyl moiety of 4-aminoacetophenone failed due to the reactivity of the unhindered aromatic amine. However, once the bromination of the ring in positions 2 and 6 was accomplished (eq. 1), the preparation of pure α-bromo derivative became feasible (eq. 2). Since the reduction of the ketone with $NaBT_4$ did not give a product of high specific activity, we were obliged to attempt the catalytic reduction of the ketone with tritium gas (eq. 4). Preliminary experiments performed on dibromoacetophenone used as a simulator showed that the catalytic reduction progressed with simultaneous

debromination. Under the same experimental conditions, clenbuterol underwent rapid dechlorination. So, we had to include in the synthesis an additional selective aromatic chlorination[4] in order to obtain the desired labelled clenbuterol (eq. 5).



EXPERIMENTAL

General

Silica gel thin-layer chromatography (t.l.c.) plates of 0.25 mm thickness were used for preparative purification of radioactive products. Radiochemical purity was determined by radioscanning of analytical t.l.c. plates developed with two different solvent systems. The amount of labelled compound for specific activity calculations was

determined by UV absorption as referred to a known standard. Ultra-violet spectra were recorded on a Gilford 2600 spectrophotometer NMR spectra were performed in $CDCl_3$ with TMS as internal standard on a Varian EM 360 spectrometer, and IR spectra were performed in $CHCl_3$ on a Perkin-Elmer 256 grating spectrometer. Radiochemical purity was determined by radiochromatogram scanning on t.l.c. plates on a Berthold Duennschicht Scanner II, LB 2722; total and specific activity were measured on a Packard Tri-Carb 1000 Liquid Scintillation Analyzer.

4-amino-3,5-dibromoacetophenone[3] - 4.4 gr (33 mmol) 4-aminoacetophenone and 8.2 gr (100 mmol) anhydrous sodium acetate are dissolved while stirring in 60 mL glacial acetic acid heated to 65^0C. After cooling to room temperature, a solution of 10.6 gr (66 mmol) molecular bromine in 10 mL acetic acid is slowly added dropwise with vigorous stirring. The progress of reaction is followed by disappearance of the brownish colour. At the end of reaction, the mixture is evaporated to dryness and the remaining slurry is taken up in 150 mL ethyl acetate. The solution is washed with three portions of 50 mL water, and the organic phase is dryed on $MgSO_4$. A solution of dry isopropanol.HCl is added to precipitate the HCl salt of 4-amino-3,5-dibromoacetophenone which is separated and washed with ethyl acetate. The resulting solid is dissolved in 50 mL 1 N NaOH solution, giving back the free base, extracted by three fractions of 30 mL ether. The combined etheral fractions are dryed on $MgSO_4$, filtered and evaporated to dryness, giving 7.0 gr (24 mmol, 72% yield) of a white solid, identified as 4-amino-3,5-dibromoacetophenone. m.p.: 171-172^{0}C
NMR $\delta(CDCl_3$, 60 MHz): 2.70 ppm (s. 3H) $-CH_3$, 5.25 ppm (m. 2H) $-NH_2$, 8.30 ppm (s. 2H) -aromatic CH.

4-amino-α,3,5-tribromoacetophenone[3] - To 2 gr (6.8 mmol) 4-amino-3,5-dibromoacetophenone dissolved in 50 mL $CHCl_3$ heated to reflux, is

slowly added 1.1 gr (7 mmol) molecular bromine in 3 mL $CHCl_3$.
The reaction mixture is refluxed for 1 hour, cooled to room temperature and filtered. The solution is evaporated to dryness and 3.0 gr (6.6 mmol, 97% yield) of 4-amino-α,3,5-tribromoacetophenone.HBr are collected. The crude product is basified with 1 N NaOH solution and the free base is extracted with 3 portions of 20 mL $CHCl_3$. The organic layer is dryed on $MgSO_4$ and evaporated to dryness, providing a dark yellow solid, m.p.: 112-115^{0}C, 2 gr, (5.5 mmol, 81% yield).
Purity control: t.l.c. on silicagel, using cyclohexane:ethyl acetate (7:3) (R_f: 0.64) as solvent system.
NMR δ($CDCl_3$, 60 MHz): 4.37 ppm (s. 2H) -$COCH_2Br$, 5.20 ppm (broad s. 2H) -NH_2, 8.25 ppm (s. 2H) -aromatic CH.

4-amino-3,5-dibromo-α-tert.butylaminoacetophenone[3] - To 1 gr (2.7 mmol) 4-amino-α,3,5-tribromoacetophenone dissolved in 10 mL $CHCl_3$ and heated to 50^{0}C are added with stirring 1.5 mL (14.4 mmol) tert.butylamine. The mixture is refluxed for 2 hours. After cooling to room temperature, the solution is made alkaline with 1 N NaOH, the organic phase is separated, dryed on Na_2SO_4, and evaporated to dryness. A light yellowish solid (m.p. 128-130^{0}C) is obtained and identified as 4-amino-3,5-dibromo-α-tert.butylaminoacetophenone (4.3 gr, 12.2 mmol, 90% yield). NMR δ($CDCl_3$, 60 MHz): 1.35 ppm (s. 9 H) -$C(CH_3)_3$, 5.15 ppm (m.2H)-NH_2, 8.55 ppm (s. 2H) -aromatic CH. IR: ν_{CO} 1605 cm^{-1}

The two following steps were first performed with nonradioactive compounds and the final products were identified prior to using radioactively labelled compounds.
Hydrogenation of 4-amino-3,5-dibromo-α-tert.butylaminoacetophenone resulted in hydrodehalogenation in addition to the reduction of the ketone to the alcohol. These transformations were observed in the modification of the IR spectrum, where ν_{CO} disappeared and ν_{CHOH} 1120, 1250 cm^{-1} appeared, and in the NMR spectra where aromatic CH

singlet at 8.55 ppm becomes doublet of doublets at 7.05 ppm.

2-(tert.butylamino)-1-(4-amino-[3,5-^{3}H]-phenyl)-[1-^{3}H]-ethanol[10] - 36 mg (0.1 mmol) of purified 4-amino-3,5-dibromo-α-tert.butylaminoacetophenone is dissolved in 1 mL dioxane and 1 mL triethylamine, and the solution is transferred to a glass ampoule provided with a magnetic stirrer bar and 40 mg Pd/C (10%). The reaction vessel is connected to a capillary vacuum manifold, the mixture is frozen (liquid nitrogen) and the vessel is evacuated. Tritium gas (600 mm Hg pressure) of 99% radiochemical purity is introduced and after reaching ambient tempera rature the reaction mixture is stirred overnight. After no further consumption of tritium is observed (no changes in the tritium pressure gauge), 16 Ci (592 GBq) tritium gas have been consumed. The residual tritium is evacuated, the reaction mixture is washed with methanol (3 x 3 mL) to remove labile tritium, and is separated from the catalyst by filtration. The solution is acidified with 2 mL 1 N HCl, evaporated to dryness, and dissolved in water. The crude tritiation product (3.5 Ci, 130 GBq) is checked by tlc using the solvent system ethyl acetate:cyclohexane (7:3) giving one main radioactive peak at R_f: 0.16 (90% of the total radioactivity). This peak corresponds to the chemical spot of a derivative prepared by the catalytic hydrogenation of an authentic clenbuterol sample.
This crude radioactive product is used without further purification in the next step.

2-(tert.butylamino)-1-(4-amino-3,5-dichlorophenyl)-[1-^{3}H]-ethanol; [ethanol-1-^{3}H]clenbuterol[4]* - An aqueous solution containing 200 mCi (7.4 GBq) of the crude product is evaporated to dryness and the residue is dissolved in 1 mL aqueous acetic acid (1:1) cooled at 0^{0}C. The reaction vessel is protected from light and a cool solution of saturated chlorine in acetic acid (1.5 mL, 30 mg Cl_2) is then added, the mixture is stirred for 30 min. at 0^{0}C.; a saturated aqueous

solution of $NaHSO_3$ is added thereafter and stirring is continued for 15 min. The reaction mixture is made alkaline with 2 N NaOH solution and extracted with chloroform (3 x 10 mL). The organic phase is dried on Na_2SO_4, filtered, evaporated to dryness and the residue is dissolved in a minimum volume of methanol, giving 55 mCi (2.04 GBq) of tritiated clenbuterol (82% radiochemical yield, taking into account the loss of 2 tritium atom equivalents due to detritiation in the chlorination reaction). The product is finally purified on silica gel plate in the solvent system ethyl acetate: acetic acid: methanol (8:1:1) and the radioactive peak corresponding to clenbuterol is extracted with benzene, giving 6.5 mCi (241 MBq) of radiochemically and chemically >99% pure product.

Purity control: by tlc in the following solvents systems: methanol: acetic acid (100:3) R_f: 0.6; methanol:ammonium hydroxide (100:1.5) R_f: 0.5; methanol:chloroform (9:1) R_f: 0.2; methanol:acetic acid:ethyl acetate (1:1:8) R_f: 0.5. Specific activity: 13.4 Ci/mmol (496 GBq/mmol).

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* according to similar chlorinations.

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