SYNTHESIS OF BENZYLIC CARBON-14 LABELLED TULOBUTEROL-HCl

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SUMMARY

Tulobuterol•HCl, a β-adrenergic bronchodilating agent, was labelled with carbon-14 in the benzylic position for metabolism studies.

Key Words: Carbon-14 labelling, Tulobuterol•HCl, Bronchodilating agent

Tulobuterol (I) is a sympathomimetic amine which is clinically effective as a β-adrenergic bronchodilator[1-3]. In an effort to determine the metabolic fate of this agent, methylene carbon-14 labelled tulobuterol (II) was synthesized[4]. However, in rat[5], guinea pig and rabbit[6] metabolism studies, a portion of the carbon-14 dose was eliminated in the expired air as radiolabelled carbon dioxide, therefore indicating that the side chain of the molecule was oxidized. To avoid the loss of radioactivity as $^{14}CO_2$ during metabolism, it was decided to label tulobuterol in a more metabolically stable position. This paper describes the synthesis of benzylic carbon-14 labelled tulobuterol (III).

Earlier synthetic work[4] consisted of labelling tolubuterol at the homobenzylic position (as in II) and this was done using carbon-14 labelled diethyl malonate as the starting material (Figure 1).

For the synthesis of III, a similar approach was adopted. The present approach, however, involved use of carbon-14 labelled barium carbonate, a

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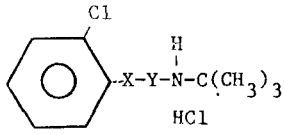

I, X = $-\underset{\underset{OH}{|}}{CH}-$; Y = $-CH_2-$

II, X = $-\underset{\underset{OH}{|}}{CH}-$; Y = $-\overset{*}{C}H_2-$

III, X = $-\overset{*}{\underset{\underset{OH}{|}}{C}}H-$; Y = $-CH_2-$

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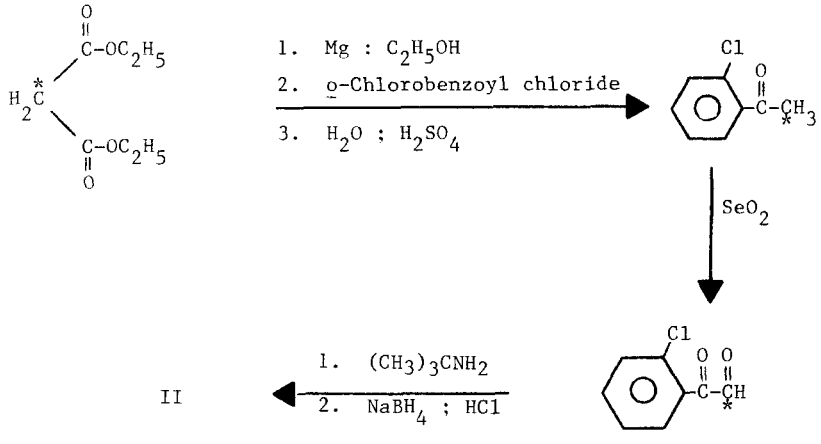
* Position of the carbon-14 label.

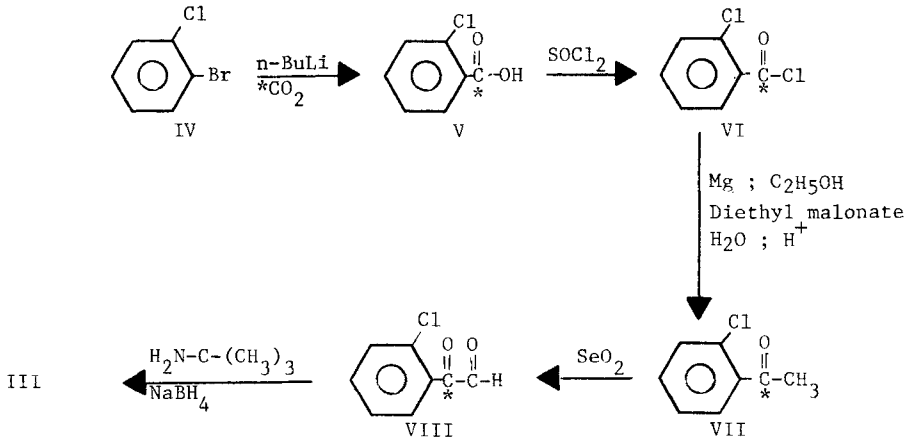
Figure 1



radiochemical that is routinely available and comparitively less expensive. Thus, following the scheme which is outlined in Figure 2, chemically and radiochemically pure tulobuterol (III) was obtained and this material was used for metabolism studies in animals.

Synthesis of o-chlorobenzoic acid-^{14}C (V) was achieved by carbonation of the lithio derivative of o-chlorobromobenzene, which was prepared according to the method described by Gilman et al[7]. The acid (V) was then converted to the acid chloride (VI) which was subseqently treated with the magnesium derivative of diethyl malonate. The resulting magnesium complex was then hydrolyzed and

Figure 2



decarboxylated in the presence of acid (the reaction mechanism involved in the hydrolysis process of the intermediate diethyl malonate derivative may be referred to as $^{A}AC^{2}$) to give o-chloroacetophenone-^{14}C (VII). Conversion of VII to the desired compound, III was then carried out using essentially the procedure used

for the synthesis of unlabelled tulobuterol[8]. In this way about 3 mCi of carbon-14 labelled (III) was obtained. This material had a specific activity of 6.62 mCi/mMole.

EXPERIMENTAL

o-Chlorobenzoic Acid-^{14}C (V)

A solution (1.3 ml; 2 mMole) of n-butyl lithium in hexane was kept at -95 to -100°. To this solution was added slowly, with stirring 5 ml solution of anhydrous ethyl ether containing 3 mMole of o-chlorobromobenzene and the flask was kept at -95 to -100° for 40 minutes with stirring. To this solution, 2 mMole of carbon-14 labelled CO_2 (resulting from about 50 mCi of carbon-14 labelled $BaCO_3$ and 210 mg of unlabelled $BaCO_3$) was then vacuum transferred and allowed to react, first at -95 to -100° for 20 minutes and then at -70° for 20 minutes. After this period, the flask was removed and kept in an ice-bath. To the cooled contents were added 2 ml of 6 N H_2SO_4 and 5 ml of H_2O, and the mixture was extracted with ether. The ether layer was separated and solvent ether was removed completely under vacuo. The residue was then treated with 10 ml of 2 N Na_2CO_3 solution and extracted with ether. The alkaline layer was separated and made acidic with 6 N H_2SO_4 and extracted with ether. The ether layer was then separated and solvent ether was removed completely under vacuo. The mass spectrum of the residue, 270 mg, was compatible with the assigned structure. MS (Low-Resolution, EI): 111, 139 (base peak), 156 ($M^{+\cdot}$). To this residue was added 860 mg of unlabelled o-chlorobenzoic acid and the entire solid was dissolved in ether. Solvent ether was then removed completely under vacuo and the residue was dried under P_2O_5. This solid weighed 1.13 gm and had a specific activity of 6.91 mCi/mMole. IR (KBr): $-\overset{O}{\overset{\|}{C}}-$ of $-\overset{O}{\overset{\|}{C}}-OH$ at 1695 cm^{-1}, benzene ring at 1595, 1572, 1480 and 750 cm^{-1}. ^1H-NMR (90 MHz; $CDCl_3$): aromatic protons at 8.1-7.2 ppm. This material was at least 98% radiochemically pure by thin-layer chromatography analyses: 1. $CHCl_3$:MeOH:conc. NH_4OH (28-30%) (60:40:2, v/v/v); and 2. $CHCl_3$:MeOH:HCO_2H (88%) (95:5:2, v/v/v).

o-Chlorobenzoyl Chloride-^{14}C (VI)

To 1.1 gm (48.78 mCi) of o-chlorobenzoic acid-^{14}C (V), 16 ml of freshly distilled $SOCl_2$ was added and the contents were heated to gentle reflux with stirring for 4 hours. After this period, about 11 ml of $SOCl_2$ was removed by slow distillation and the remaining $SOCl_2$ was removed using dry benzene (2 x 20 ml) to give VI as an oily residue.

o-Chloroacetophenone-^{14}C (VII)

Under nitrogen, to 165 mg of magnesium in 3 ml of absolute ethanol, 300 µl of CCl_4 was added and the mixture was heated to gentle reflux for 1 hour. After this period, a solution of 1 gm of diethyl malonate in 6 ml of absolute ethanol and 3 ml of anhydrous ethyl ether was added dropwise and the mixture was heated to gentle reflux for 3 hours. After this period, a solution of acid chloride (VI) in 8 ml of anhydrous ethyl ether was added dropwise and the contents were heated to gentle reflux for 2 hours. The reaction mixture was cooled and shaken with H_2SO_4 until all the solid dissolved. The ether phase was separated and the aqueous layer was extracted with an additional 50 ml of ether. The ether extracts were combined and washed with water and the solvent was removed by slow distillation. To the crude residue thus obtained was added a solution of 15 ml glacial acetic acid and 1.5 ml of conc. H_2SO_4 and 12 ml of water and the mixture was heated under reflux for 4 hours. The reaction mixture was then chilled in an ice-bath, made alkaline with 25% NaOH solution and extracted with several portions of ether. The combined ethereal extracts were then washed with H_2O and dried with anhydrous Na_2SO_4, and the solvent was removed by slow distillation to give an oily residue. Mass spectrum of this residue indicated formation of the desired substance. MS (Low-Resolution, EI): 111, 139 (base peak), 154 ($M^{+\cdot}$). This material was found to be at least 99% radiochemically pure by thin-layer chromatography analysis using $CHCl_3$ as the solvent.

o-Chlorophenyl-glyoxal-^{14}C (VIII)

To a heated solution (at 50-60°) of 1.3 gm of SeO_2 in 50 ml of p-dioxane and 0.3 ml of H_2O was added VII and the contents were heated to gentle reflux with stirring for 4 hours. After this period, the resulting mixture was filtered and the solvent from the clear filtrate was removed completely under vacuo when a residue was obtained. To this residue, 150 ml of ethyl ether was added and the ether layer was separated. This ether layer was then washed with H_2O and dried with anhydrous Na_2SO_4. It was then filtered and solvent ether was removed completely under vacuo. The mass spectrum of this material, VIII, indicated formation of the desired substance. MS (Low-Resolution, EI): 111, 139 (base peak), 168 ($M^{+\cdot}$), 169.

o-Chloro-α-(tert-butylaminomethyl)benzyl alcohol HCl-^{14}C (III)

The residue, VIII, was dissolved in 20 ml of dry methanol and to it a solution of 1.6 gm of t-butylamine in 10 ml of dry methanol was added at room temperature and the contents were stirred under nitrogen for 45 minutes. After this period, it was cooled in an ice-bath and 2 x 250 mg of $NaBH_4$ was added. Ice-bath was then removed and the contents were stirred at room temperature under nitrogen for 18 hours. After this period, the solvent was removed under vacuo and the residue was dissolved in 50 ml of 15% HCl solution. This acidic layer was washed with ether and made alkaline with K_2CO_3 solution and extracted with ether. The ether layer was then washed with water and solvent ether was removed completely under vacuo. The residue thus obtained, was dissolved in 23 ml of 10% HCl solution. This acidic solution was lyophilized and the residue was purified by ion-exchange column chromatography. This purification was carried out using Amberlite CG-50 resin (200-400 mesh) in the NH_4^+ form. Elution was done successively with 0.2, 0.5 and 1% NH_4OH solution. Fractions containing the desired substance were combined and this solution was lyophilized. The residue obtained after lyophilization was dissolved in 10 ml of 5% HCl solution, filtered through 0.45 μm filter and the clear solution was lyophilized to give a gummy residue. This residue was then triturated with anhydrous ether when

a crystalline solid separated. It was collected on a filter and dried to give 116 mg of III.

IR (KBr): OH at 3350 cm^{-1}, amine salt at 2700-2300 cm^{-1}, benzene ring at 1575, 1480 and 760 cm^{-1}. ^{1}H-NMR (90 MHz, CD_3OD): aromatic protons at ~7.8-7.2 ppm, -$\underline{C}$H-OH at 5.4 ppm, -$\underline{CH_2}$N- at ~3.1 ppm, -C$(CH_3)_3$ at 1.4 ppm. ^{13}C-NMR (22 MHz, CD_3OD): benzene ring at 128.7 (2 carbons), 130.6, 130.8, 132.7 and 139.8 ppm, -$\underline{C}$H-OH at 67.7 ppm, $-CH_2-$ at 58.4 ppm, $-(CH_3)_3$ at 25.8, -$\underline{C}(CH_3)_3$ not visible. MS (Low-Resolution, EI): 86 (base peak), 194, 212, 227 ($M^{+\cdot}$), 228. This analysis indicated about 10.7% incorporation of the carbon-14 at the desired position in the molecule.

DETERMINATION OF CHEMICAL AND RADIOCHEMICAL PURITY AND IDENTITY OF TULOBUTEROL-HCl (III)

Thin-Layer Chromatography

A sample of III was spotted on Silica Gel GF plates (250 μ) which were developed in the solvent systems listed below:

Solvent System	Rf	% Radiochemical Purity
1. $CHCl_3$:MeOH:NH_4OH (28-30%) (75:20:2, v/v/v)	0.85	99.75
2. Dichloromethane:MeOH:gl. AcOH (60:40:2, v/v/v)	0.83	99.76
3. $CHCl_3$:MeOH:HCO_2H (88%) (60:40:2, v/v/v)	0.79	99.66
4. EtOAc:MeOH:Triethylamine (50:50:5, v/v/v)	0.83	99.35
5. n-Hexane:MeOH:NH_4OH (28-30%) (20:80:2, v/v/v)	0.88	98.23

The silica gel was sequentially scraped from each plate and placed into vials containing 10 ml of Instagel Cocktail and the radioactivity was determined by scintillation counting. The results, based on the distribution of radioactivity along the length of the chromatographic plates indicated that tulobuterol-HCl (III) was at least 98% radiochemically pure.

High-Pressure Liquid Chromatography Radioanalysis

The liquid chromatographic analysis was performed using Waters Associates Liquid Chromatograph Model #6000A equipped with Rheodyne injector. The absorption medium was Waters μ-Bondapak/C_{18} (4 mm x 30 cm) and DuPont Instrument UV spectrophotometer (wavelength 262 nm) was used for detection. Solvent system containing 1.4 gm of Na-lauryl sulfate, 10 ml of acetic acid, 320 ml of H_2O and 700 ml of MeOH was used for elution. The flow rate was 1.5 ml/min and 1500 p.s.i. pressure was obtained. Under these conditions, I and III showed retention times of 4.24 and 4.21 minutes, respectively. In order to establish radiochemical purity using high-pressure liquid chromatography, fractions of the effluent (0.75 ml/0.5 min) were collected directly in vials containing 10 ml of Instagel and the radioactivity was determined by scintillation counting. The results of this analysis indicated that III was at least 99% radiochemically pure. When an aliquant of the solution used in this analysis was placed directly into the vial and counted under identical conditions, the recovery of radioactivity from the column was found to be 102%.

Chemical Assay

A sample of III was analyzed using high pressure liquid chromatography and conditions described earlier. This assay indicated that this material was about 97% chemically pure.

Ultraviolet Absorption Spectroscopy

The samples of I and III, prepared in methanol, exhibited absorption at 262.8 nm and 261.2 nm respectively, thereby indicating their chemical identity.

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