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SYNTHESIS OF [^{14}C]CILADOPA

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SUMMARY

[^{14}C]Ciladopa (S(-)-2-[4-[[2-^{14}C]-2-hydroxy-2-(3,4-dimethoxyphenyl)ethyl]-1-piperazinyl]-2,4,6-cycloheptatrien-1- one hydrochloride; AY-27,110 hydrochloride) has been synthesized in six steps incorporating [^{14}C]carbon dioxide. [7-^{14}C]Acetoveratrole, obtained from veratric acid via the acid chloride, was brominated and coupled with a troponylpiperazine salt. The resulting ketone was stereospecifically reduced microbiologically to give the S(-) enantiomer of the corresponding alcohol. Two batches of [^{14}C]ciladopa were produced, giving a combined overall yield of 25% from [^{14}C]barium carbonate (sp. act. 44.7 ± 0.6 and 43.4 ± 0.8 μCi/mg; 99.2 and 98.9 % radiochemical purity, respectively).

Key words: Dopamine agonists, ciladopa, AY-27,110 hydrochloride, ^{14}C

INTRODUCTION

Ciladopa belongs to a chemically novel class of dopamine receptor agonists (1). To study the metabolic disposition of ciladopa in laboratory animals, a synthesis of the [^{14}C]labelled compound was undertaken as shown in Scheme I:

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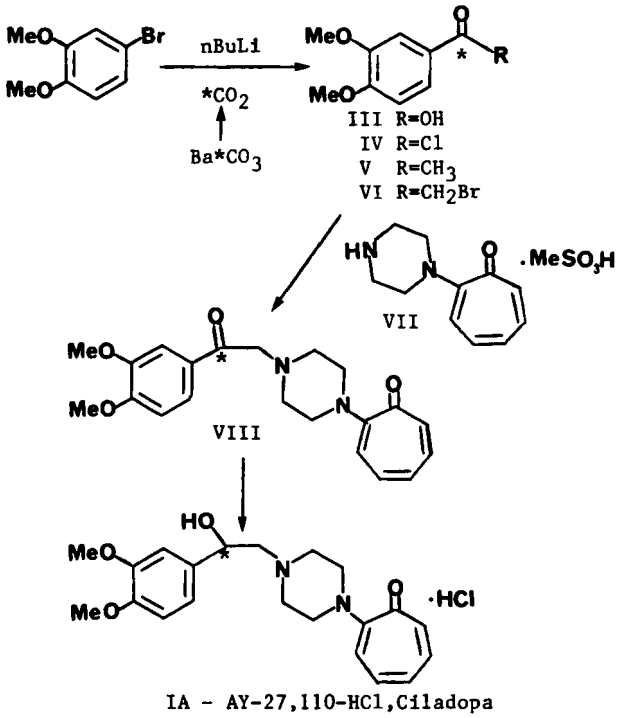
Scheme 1



IA - AY-27,110-HCl,Ciladopa
IB - AY-27,109-HCl

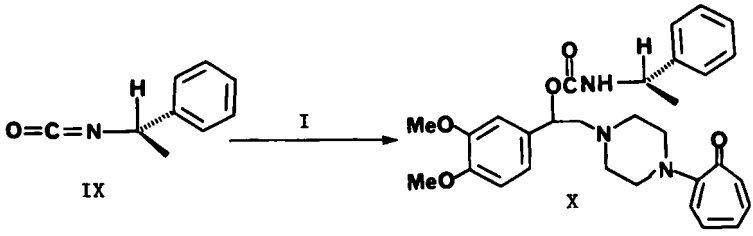
*position of ^{14}C label

DISCUSSION

Veratryllithium, prepared by the reaction of 4-bromoveratrole (II) with n-butyllithium, was carbonated (2) with [^{14}C]carbon dioxide liberated from [^{14}C]barium carbonate in 89% yield. The acid chloride IV, obtained on refluxing III in thionyl chloride, was reacted with dimethyl copper lithium (3) to give [^{14}C]acetoveratrole (V). Bromination of V with cupric bromide (4) gave VI, which was slightly contaminated with the dibromo compound. Addition of the troponyl piperazine VII to VI gave the ketone VIII in 47% yield after purification by column chromatography.

The dopamine agonist activity of ciladopa has been shown to reside in the S(-) enantiomer (1). Since chemical methods of enantiomer resolution rarely result in

compounds of absolute enantiomeric purity, the crude ketone VIII was stereospecifically reduced microbiologically with Candida guilliermondii. The optical purity of the [^{14}C]ciladopa was determined by HPLC. The labelled compound was reacted with (S)-(-)-(α)-methylbenzyl isocyanate (IX), and the resulting diastereomeric carbamates X were separated by HPLC. No residual R(+)-AY-27,109 (IB) could be detected in the [^{14}C]ciladopa, indicating that the reduction was completely stereospecific.



IX X

The [^{14}C]ciladopa was obtained in an overall radiochemical yield of 25% based on [^{14}C]barium carbonate: 0.214 g, 44.7 μCi/mg and 0.212 g, 43.4 μCi/mg. The radiochemical purity was determined to be 99.2 and 98.9%, respectively, by TLC autoradiography in three solvent systems.

EXPERIMENTAL

The synthesis of [^{14}C]ciladopa was begun with [^{14}C]barium carbonate (75 mCi, sp. act. 59.0 mCi/mmole) purchased from New England Nuclear, Boston, Massachusetts. The intermediates in the synthesis were characterized in trial experiments with unlabelled material. The reactions in the labelled synthesis were monitored by TLC using unlabelled reference compounds.

[7-^{14}C]Veratric acid (III)

The carbonation apparatus comprised a high-vacuum manifold equipped with attachments to a reaction flask, a carbon dioxide generator, a mercury manometer, and a dry nitrogen inlet-outlet. The carbon dioxide generator consisted of a round bottomed flask, containing [^{14}C]barium carbonate (0.249 g, 75 mCi, 1.26 mmole) and

cold barium carbonate (0.478 g, 2.43 mmole) and fitted with a pressure equalizing dropping funnel charged with concentrated sulfuric acid (11 ml). The generator was attached to the manifold by a drying tube containing anhydrous calcium sulfate.

The system was filled with dry nitrogen, and the reaction flask was charged with dry ether (25 ml) and 4-bromoveratrole (2.40 g, 11.1 mmole). The mixture was cooled to -60°, and n-butyllithium (2.6 M in hexane) (2.8 ml, 7.28 mmole) was added. After 15 min at -60°, the reaction flask was placed in a bath of liquid nitrogen, and the system was evacuated to 0.02 mm of mercury pressure. The reaction flask was isolated from the pump, thawed to -60°, re-frozen in liquid nitrogen, and evacuated to a pressure of 0.02 mm of mercury. The lithiated bromoveratrole was carbonated at -60° by dropping the sulfuric acid slowly onto the barium carbonate. The last of the [^{14}C]carbon dioxide was drawn into the raction flask by warming the generator flask with warm water and cooling the reaction flask in liquid nitrogen. The stopcock to the generator was closed, and the reaction mixture was stirred at -60° for 30 min to complete the reaction.

Nitrogen was introduced into the system, and the reaction mixture was quenched by the addition of concentrated hydrochloric acid (5 ml). The acidic solution was extracted with ether (3 x 60 ml), and the combined ether layers were extracted with a dilute sodium hydroxide solution (3 x 60 ml). The alkaline extracts were combined, cooled in ice water, acidified, and extracted with ether (3 x 60 ml). The ether extracts were combined, dried over magnesium sulfate, and evaporated to dryness. The product was a white solid (0.596 g, 89%).

[7-^{14}C]Veratroyl chloride (IV)

The carboxylic acid III (0.596 g, 3.30 mmole) and thionyl chloride (12 ml) were refluxed for 4 hr. The reaction mixture was repeatedly dissolved in benzene and evaporated to dryness to remove the excess thionyl chloride. The crude acid chloride was obtained as a yellow brown solid (0.647 g, 99%) and was used in the next reaction without further purification.

[7-^{14}C]Acetoveratrole (V)

Cuprous iodide (1.95 g, 10.3 mmole) was suspended in dry ether (70 ml) and cooled to 0° in an ice bath. Methyllithium (1.4 M in ether) (14.7 ml, 20.6 mmole) was added, and the reaction was stirred at 0° for 30 min, then cooled to -68°. The crude acid chloride (0.647 g, 3.23 mmole) dissolved in dry ether (25 ml) was added dropwise. The mixture was stirred at -68° for 1 hr, poured into an ice cold ammonium chloride solution (100 ml), and extracted with ether (3 x 150 ml). The ether layers were washed successively with an ammonium chloride solution (100 ml), a sodium bicarbonate solution (100 ml), and water (100 ml), combined, dried over magnesium sulfate; and evaporated to dryness. The residue, a yellow brown solid (0.528 g, 91%), was homogeneous on TLC (R_f = 0.42; chloroform).

[7-^{14}C]-α-Bromoacetoveratrole (VI)

The [^{14}C]acetoveratrole (0.528 g, 2.94 mmole) was dissolved in chloroform (7 ml) and ethyl acetate (7 ml) and heated to reflux. Cupric bromide (1.31 g, 5.89 mmole) was added, and the reaction was stirred and refluxed for 1.5 hr. The mixture was filtered through a pad of celite, and the filter cake was washed with chloroform. The filtrate and washings were combined and evaporated to dryness. The residue (0.811 g) exhibited one major spot on TLC (R_f = 0.51; chloroform) along with trace amounts of the dibromo derivative (R_f = 0.62) and starting material (R_f = 0.40). The crude product was purified by flash chromatography on silica gel with ethyl acetate-hexane (1:4) as the solvent. When combined and concentrated to dryness, the fractions containing the desired bromide afforded a white solid (0.568 g, 75%).

2-[4-[[2-^{14}C]-2-Oxo-2-(3,4-dimethoxyphenyl)-ethyl]-1-piperazinyl]-2,4,6-cycloheptatrien-1-one (VIII)

The bromide (0.568 g, 2.19 mmole) and potassium carbonate (0.667 g) were stirred and refluxed in acetonitrile (30 ml). Troponyl piperazine methane sulfonate (VII) (0.628 g, 2.20 mmole) was added in portions over a period of 45 min. The reaction

was stirred and refluxed for 1 hr and then evaporated to dryness. The residue was taken up in chloroform, washed with water, dried over magnesium sulfate, evaporated to dryness, and chromatographed on a silica gel column with ethyl acetate as solvent. When combined and evaporated to dryness, the fractions containing the desired product afforded a yellow solid (0.498 g; 62%).

(-)2-[4-[[2-^{14}C]-2-Hydroxy-2-(3,4-dimethoxyphenyl)ethyl]-1-piperazinyl]-2,4,6-cycloheptatrien-1-one hydrochloride(ciladopa) (IA)

Resting cells of Candida guilliermondii (25 g) were suspended in phosphate buffer (250 ml, pH = 5.0) in a sterilized 2 litre erlenmeyer flask. The ketone VIII (0.498 g, 1.35 mmole) was added in portions and evenly suspended in the mixture. A sterile 50% glucose solution (6 ml) was added, and the flask was shaken at 37° for 90 hr. The pH of the mixture was adjusted to 3 by the addition of 2 N hydrochloric acid, and the mixture was filtered through celite. The celite cake was washed with slightly acidic water. The pH of the filtrate was adjusted to 8-9 by the addition of a 10 N sodium carbonate solution. The mixture was extracted with methylene chloride (4 x 150 ml). The organic extracts were combined, dried over anhydrous sodium sulfate, and concentrated to dryness to give a light yellow foam (0.460 g, 92%).

The crude [^{14}C]ciladopa free base was dissolved in methanol (0.8 ml) and divided into two portions in two Craig tubes. Concentrated hydrochloric acid (0.06 ml) was added, and the [^{14}C]ciladopa was crystallized by the addition of ether. The crystals were collected by centrifugation, recrystallized from methanol, collected and dried under vacuum at 60°, toyield two batches of [^{14}C]ciladopa (0.214 g, 44.7 μCi/mg and 0.212 g, 43.4 μCi/mg).

The radiochemical purity of [^{14}C]ciladopa was determined by TLC autoradiography in three solvent systems: (a) methylene chloride: acetone: triethylamine = 30:10:0.6; (b) acetonitrile: methanol: ammonium hydroxide = 34:4:1; and (c) ethyl acetate: methanol: triethylamine = 28:12:0.4. The radioactive zones were located by exposing the TLC plates to Kodak XAR Medical X-ray film. The silica gel (1 cm

sections) was scraped into counting vials, digested in water (0.2 ml) and 50% hydrofluoric acid (0.2 ml), and counted in Aquasol scintillation cocktail (15.0 ml).

Analysis of the [^{14}C]ciladopa by HPLC indicated that no residual R(+)-AY-27,109 (IB) was present. The TLC, MS, IR, and NMR properties of the [^{14}C]ciladopa were identical to those of an authentic sample of ciladopa: IR (KBr) 3420, 1565, 1260, 1140 cm^{-1}; NMR (DMSO - d_6) 7.3 - 6.6 (8H, m, aromatic), 4.7 (1H, b_2, OH), 3.92 (3H, 2, OCH_3), 3.88 (3H, 2, OCH_3), 3.4 (4H, m, CH_2N), 2.8 (7H, m, CH_2N, CHOH); MS m/e 370 (M+).

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