

SYNTHESIS OF BISARAMIL LABELLED WITH CARBON-14 AND DEUTERIUM

J. Szammer, E. Simon-Trompler, S. Mlinko

Central Research Institute for Chemistry of the Hungarian
Academy of Sciences; H-1525 Budapest, PO.Box 17, Hungary

SUMMARY

[6,8- $^{14}\text{C}_2$]-Bisaramil : 3-methyl-7-ethyl-9 α -(4-chlorobenzoyloxy)-3,7-diazabicyclo/3.3.1./nonane-[6,8- $^{14}\text{C}_2$] monohydrochloride and [7-N-D $_5$ -ethyl]-Bisaramil : 3-methyl-7-[D $_5$ -ethyl]-9 α -(4-chlorobenzoyloxy)-3,7-diazabicyclo/3.3.1./nonane monohydrochloride were synthesized in four steps from $^{14}\text{CO}_2$ (6 % overall yield) and in six steps from [D $_6$]-ethanol (4 % overall yield), respectively.

Key words : carbon-14, deuterium, Bisaramil, antiarrhythmic agents

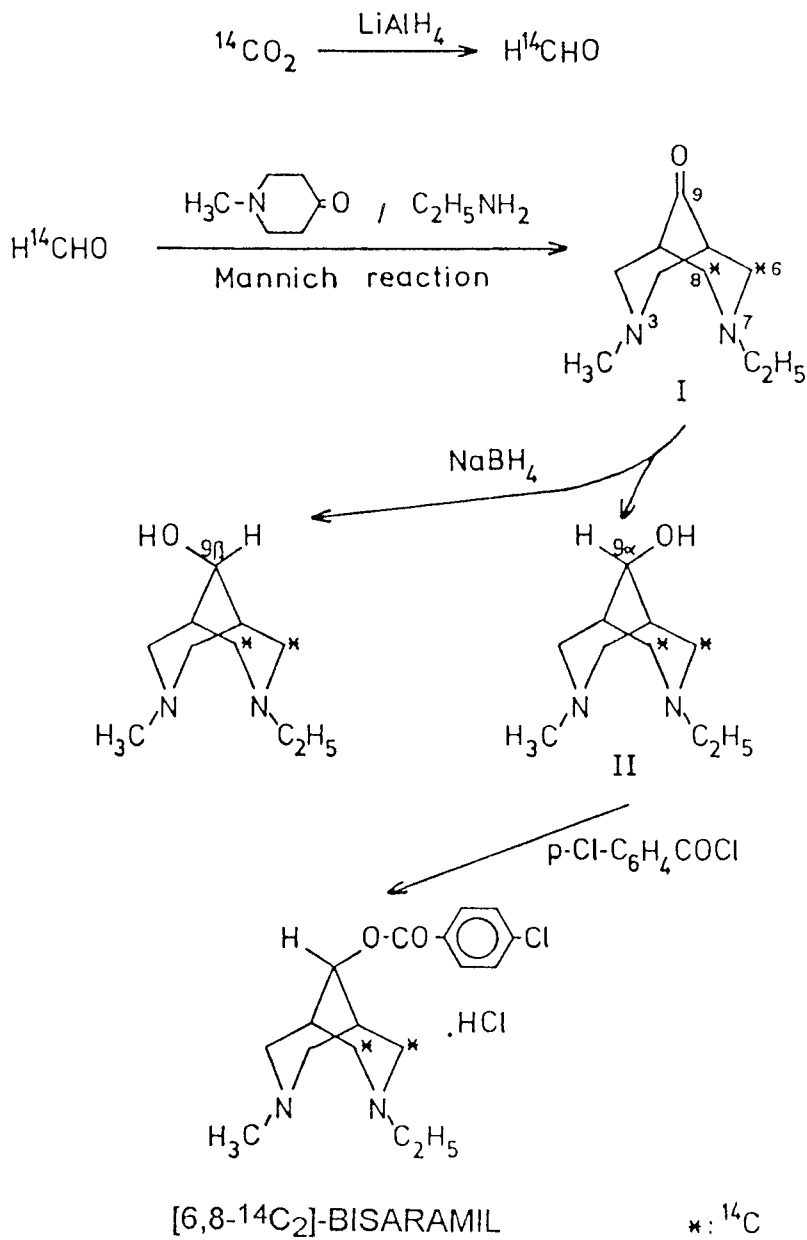
INTRODUCTION

Some derivatives of azabicyclo/3.3.1./nonanes⁽¹⁻³⁾ possess good antiarrhythmic activity^(3,4). Among these Bisaramil⁽⁵⁾ is a drug (Yutac®) used in the treatment of antiarrhythmic diseases. Carbon-14 and deuterium labelled Bisaramil was synthesized for pharmacokinetic and metabolic studies.

DISCUSSION

[6,8- $^{14}\text{C}_2$]-Bisaramil was synthesized according to the scheme 1. The two labelled positions are suitable for metabolic studies and afford the possibility to achieve higher specific activities.

For biological studies Bisaramil containing more than three deuterium atoms in the molecule was required. [7-N-D $_5$ -ethyl]-Bisaramil,



SCHEME 1

synthesized according to scheme 2, is suitable for this purpose.

The solubility and sorption properties of deuterated and undeuterated Bisaramil have significant differences. Thus, the R_f - values and the retention times of Bisaramil and [7-N-D₅-ethyl]-Bisaramil were respectively 0.6 and 0.5 in TLC and 14.8 min and 16.4 min in HPLC analysis (see below).

EXPERIMENTAL SECTION

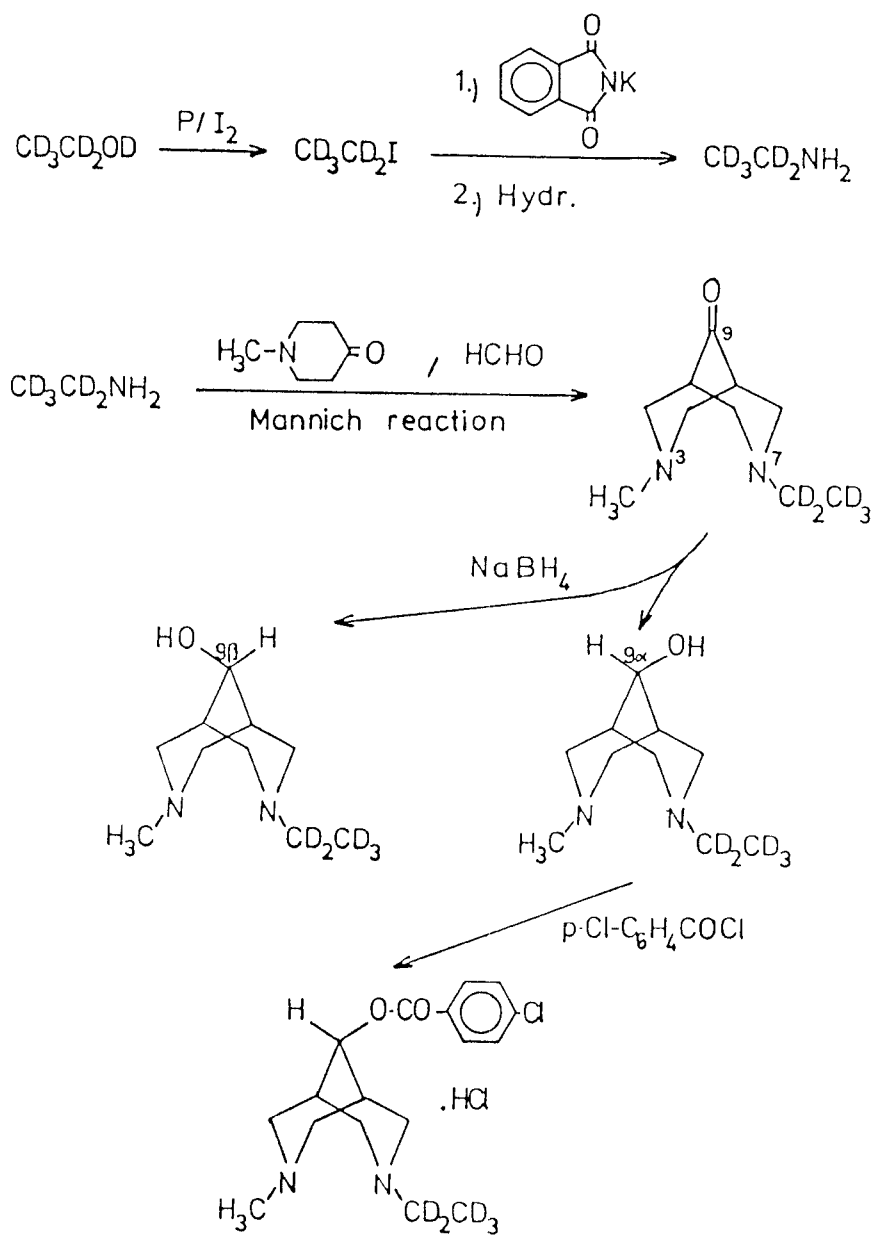
[¹⁴C]-Formaldehyde

The reduction of ¹⁴CO₂ liberated from Ba¹⁴CO₃ (1 g, 5.2 GBq), was performed in 10 ml of tetrahydrofuran containing 0.16 g of LiAlH₄ at -30 °C for one hour. The reaction mixture was hydrolysed with dilute H₂SO₄. Distillation was effected in a slow stream (3 L/h) of nitrogen, employing a water trap in the receiver. The fraction boiling at 80 - 100 °C contained the main part of [¹⁴C]-formaldehyde. The predistillate (solvent) and the residue were diluted and further distilled two times with 4 ml of water, containing 30 mg of formaldehyde. The [¹⁴C]-formaldehyde content and its radioactivity were determined respectively from an aliquot part of the solution by an iodometric method and the radioactivity of part of the solution by an iodometric method and the radioactivity of the dimedone derivative. Yield was 3.5 mmol (1.80 GBq).

3-Methyl-7-ethyl-9-oxo-3,7-diazabicyclo/3.3.1./nonane-[6,8-¹⁴C₂] (I)

Place 0.32 ml of a solution of ethylamine (24.5 % in methanol) and 2 ml of methanol in a 100 ml round-bottomed flask fitted with a reflux condenser, magnetic stirrer and a dropping funnel. Cool the mixture to 0 °C, and add 0.11 ml of acetic acid, then 10 ml of methanol. Heat the mixture to reflux, and add in three portions of "A" and "B" solutions at intervals of 30 minutes.

Solution "A": Acetic acid (0.1 ml) was added to 0.19 g of N-methyl-4-piperidon in 20 ml of methanol.

[7-N-D₅-ETHYL]-BISARAMIL

SCHEME 2

Solution "B": Water solution of [¹⁴C]-formaldehyde (12 ml) diluted with 2 ml of methanol.

After addition of the third portions of "A" and "B" solutions, the reaction mixture was heated for an additional hour, then evaporated on a rotary evaporator. The residue was column chromatographed on 30 g silica gel (solvent : ethyl acetate : isopropanol : ammonia = 2 : 2 : 1). Yield : 0.218 g. Radioactivity : 1.1 GBq.

3-Methyl-7-ethyl-9 α -hydroxy-3,7-diazabicyclo[3.3.1]nonane-[6,8-¹⁴C₂]

dihydrochloride (II)

To 0.218 g of (I) were added 2 ml of methanol and 0.030 g of NaBH₄ dissolved in 0.6 ml of 0.1 N NaOH. Reduction took place immediately. After evaporation of the solvent, the residue was extracted four times with 1 ml of chloroform, dried over anhydrous MgSO₄ and the solvent was removed on a rotary evaporator. The residue was acidified with HCl/isopropanol to pH 1 and evaporated to dryness. The resulting dihydrochloride of α - and β -isomers was extracted six times with 2 ml of hot abs. isopropanol to dissolve the β -isomer. After these extractions the white crystalline precipitate contained more than 98 per cent of α -isomer. The yield was 0.130 g.

[6,8-¹⁴C₂]-Bisaramil

To a solution of (II) : 0.130 g in 1.5 ml of pyridine, 0.20 g of 4-chlorobenzoyl chloride was added. The reaction mixture was heated at 80 °C for one hour and further heated for two hours after addition of 1 ml of abs. ethanol. The solvents were evaporated on a rotary evaporator. The raw product was purified by column chromatography, using 30 g of silica gel and ethyl acetate : isopropanol : ammonia = 2 : 2 : 1 as eluent. The pure fractions were combined and evaporated. The residue was dissolved in 1 ml of abs. isopropanol and acidified with HCl/abs. isopropanol to pH 6.5 and evaporated to dryness. The product was crystallized in the following way : 5 ml of chlorobenzene was added to the solution of the residue in 0.4 ml of abs. isopropanol. The mixture

was slowly distilled under normal pressure until the onset of crystallisation, allowed to cool to room temperature for some hours. The crystals were filtered, washed with a small quantity of abs. acetone then with ether. Yield : 0.150 g. Radioactivity : 310 MBq.

Analysis of [6,8- $^{14}\text{C}_2$]-Bisaramil

Chemical and radiochemical purity of [6,8- $^{14}\text{C}_2$]-Bisaramil was determined by TLC and HPLC methods.

Thin layer chromatography

TLC-sheet : DC-Aufoilen Kieselgel 60 F₂₅₄ (Art. 5554)

Eluent : ethyl acetate : isopropanol : 25 % ammonia sol. = 4 : 4 : 1.5

High-performance liquid chromatography

ISCO HPLC-system was used under the following conditions :

Column : Supersil silica (5 μm , 250 x 4 mm ID)

Mobile phase : mixture of 100 ml dichloroethane, 60 ml isopropanol, 16 ml chloroform and 5 ml 25 % ammonia solution

Flow rate : 1.0 ml/min ; Detection : 254 nm

On the basis of comparison Bisaramil standard and [6,8- $^{14}\text{C}_2$]-Bisaramil samples, the chemical and radiochemical purity of [6,8- $^{14}\text{C}_2$]-Bisaramil proved to be greater than 98 %.

[D₅]-Ethyl-amine

[D₅]-Ethyl iodide

Place 1.2 g of purified red phosphorus and 5 g of [D₅]-ethyl alcohol (Stohler; D₅-content > 99 %) in a 25 ml round-bottomed flask fitted a magnetic stirrer and a separatory funnel connected to a reflux condenser. The mixture was gently refluxed and 12.1 g iodine was slowly washed into the reaction mixture from the separatory funnel by means of condensed liquid. After addition of the total amount of iodine, the heating was continued for an hour. The condenser was adjusted for

distillation and most of the crude product was distilled. The receiver was cooled with a mixture of aceton and dry ice. The crude [D₅]-ethyl iodide was washed successively with water, 5 per cent sodium carbonate, 5 per cent sodium bisulfite and water. The product was dried over P₂O₅ and distilled with liquid nitrogen under vacuum. The yield was 9.6 g.

[D₅]-Ethyl-amine

Potassium phthalimide (11.5 g), 9.6 g of [D₅]-ethyl iodide and 60 ml of dimethyl-formamide were placed in a 100 ml round-bottomed flask fitted with a reflux condenser and magnetic stirrer and the stirred suspension was heated at 160 °C for 4 hours. The solvent is distilled on a rotary evaporater and the residue was treated with 60 ml of water and filtered. Air-dried [D₅]-ethyl-phthalimide (11 g) was added to the solution of ethyl alcohol (300 ml) and hydrazine hydrate (2.9 ml). The mixture was heated at 80 °C and 17 ml of dilute (1 : 1) hydrochloric acid was added and after heating for 20 minutes the solvent was evaporated on a rotary evaporater. The residue was treated with 80 ml of methanol, filtered from the precipitated phthaiyl hydrazine and the solution was rendered alkaline with an excess of sodium hydroxide solution. The liberated amine was distilled with methanol into a receiver, cooled with a mixture of aceton and dry ice. The yield was 25 mmol determined by titration from an aliquot part of the solution.

[7-N-D₅-Ethyl]-Bisaramil

The synthesis of 3-methyl-7-[D₅]-ethyl-9-oxo-3,7-diazabicyclo/3.3.1/nonane from [D₅]-ethyl-amine, its reduction to 3-methyl-7-[D₅]-ethyl-9 α , β -hydroxy-3,7-diazabicyclo/3.3.1/nonane and esterification with 4-chlorobenzoyl-chloride were performed as described for ¹⁴C-labelled compounds.

The resolution of α , β -isomers required ten-fold extraction with hot isopropanol to produce the α -isomer containing 2 per cent β -isomer. At the same time crystallisation of [7-N-D₅-ethyl]-Bisaramil containing 3 per cent of β -isomer from chlorobenzene / isopropanol results in a product without β -isomer.

Yield was 1.38 g (4 % based on [D₆]-ethanol).

Analysis of [7-N-D₅-ethyl]-Bisaramil

Chemical and isotopical purity of [7-N-D₅-ethyl]-Bisaramil was checked by TLC, HPLC and MS methods.

Thin layer chromatography and high-performance liquid chromatography

Conditions were the same as at the measurements for [6,8-¹⁴C₂]-Bisaramil.

	TLC R _f -values	HPLC Retention time
Bisaramil standard	0.6	14.8 min
[7-N-D₅-ethyl]-Bisaramil	0.5	16.4 min

Mass spectrometry

There were 70 eV mass spectra taken.

Mass spectrometer: AEI MS-902 type, direct inlet system, source temperature 150 °C

On the basis of comparison of Bisaramil standard and [7-N-D₅-ethyl]-Bisaramil samples, the purity and D₅-content of the [7-N-D₅-ethyl]-Bisaramil samples proved to be greater than 99 %.

ACKNOWLEDGEMENT

The authors wish to thank Dr. J. Tamás for mass spectra. This work was partially supported by grants N^o 1753 provided by the Hungarian Research Found (OTKA) and Chemical Works of Gedeon Richter Ltd.

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