Journal of Labelled Compounds — January-March — Vol. VII, No. 1

Labelling of 1-benzyl-1-(3'-dimethylaminopropoxy)-cycloheptane fumarate (active substance of the drug Halidor®) with ^{14}C isotope

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Received August 11, 1970.

SUMMARY

In order to study the pharmacological properties and mode of action of 1-benzyl-1-(3'-dimethylaminopropoxy)-cycloheptane fumarate its labelling was performed with ^{14}C. The radioactive carbon has been introduced into the benzyl group and into the dimethylamino moiety of the dimethylaminopropoxy side chain. The chemical and radiochemical purity of the two isotopic isomers was proved by thin layer chromatography and autoradiography.

INTRODUCTION.

In order to study the pharmacological properties and mode of action [1] of 1-benzyl-1-(3'-dimethylaminopropoxy)-cycloheptane furamate * this substance was labelled with radioactive carbon (the commercial name of this pharmaceutical product is Halidor®). The ^{14}C isotope was introduced into the benzyl substituent (VIIIa) of the molecule or into the dimethylamino group of the dimethylaminopropoxy side chain (VIIIb). Chemically and radiochemically homogeneous end products of high specific activity were obtained by the semi-micro-scale synthesis of both radioisomers with an excellent isotope utilization.

Halidor was first synthesized by Pallos, Zólyomi and Budai [2]. The drug is a spasmolytic agent with musculotropic action, suitable for arrest of spastic deformations. In addition to the vasodilator effect Halidor acts as

* The name Halidor is registered trade mark (registered in Hungary under No. 273,704 in 1963).

a minor tranquillizing and local anaestetic agent, and has also a significant coronary dilatant activity [3].

The synthesis of the drug labelled in the benzyl group was accomplished from cycloheptanone as starting material. Grignard reagent was prepared from bromobenzene and the radioactive benzoic acid obtained by carbonation with $^{14}CO_2$ was transformed into the methyl ester. The methyl benzoate was reduced by lithium aluminium hydride to benzyl alcohol. This substance was chlorinated with thionyl chloride, then the radioactive Grignard compound obtained from the benzyl chloride was reacted with cycloheptanone to give 1-benzylcycloheptanol (VI) which was condensed in turn with dimethylaminopropyl chloride. The base (VIIa) resulting from this reaction was converted into its acid fumarate (VIIIa) with fumaric acid. A radiochemical yield of 40 % based on $Ba^{14}CO_3$ was reached.

Labelling of the dimethylaminopropoxy side chain was carried out by alkylation of 1-benzyl-1-(3'-methylaminopropoxy)-cycloheptane (X) with radioactive methyl iodide. Compound X was prepared by the interaction of non-radioactive Halidor base (VII) with ethyl chloroformate followed by alkaline hydrolysis [4]. The tertiary base VIIb was separated by means of absorption chromatography from the mixture of secondary, tertiary and quaternary bases obtained by alkylation. From the radioactive base VIIb an acid fumarate (VIIIb) was prepared in the same manner as described above. The radioactive methyl iodide was utilized with a yield of 42 % in the course of these procedures. The remaining radioactivity was contained in the quaternary compound resulting from the alkylation.

Purity of the substances was checked by using paper and thin-layer chromatographic methods. The chromatograms were evaluated autoradiographically and radiometrically. The activity of the radioactive compounds was determined using a Tri-Carb liquid scintillation spectrometer.

EXPERIMENTAL.

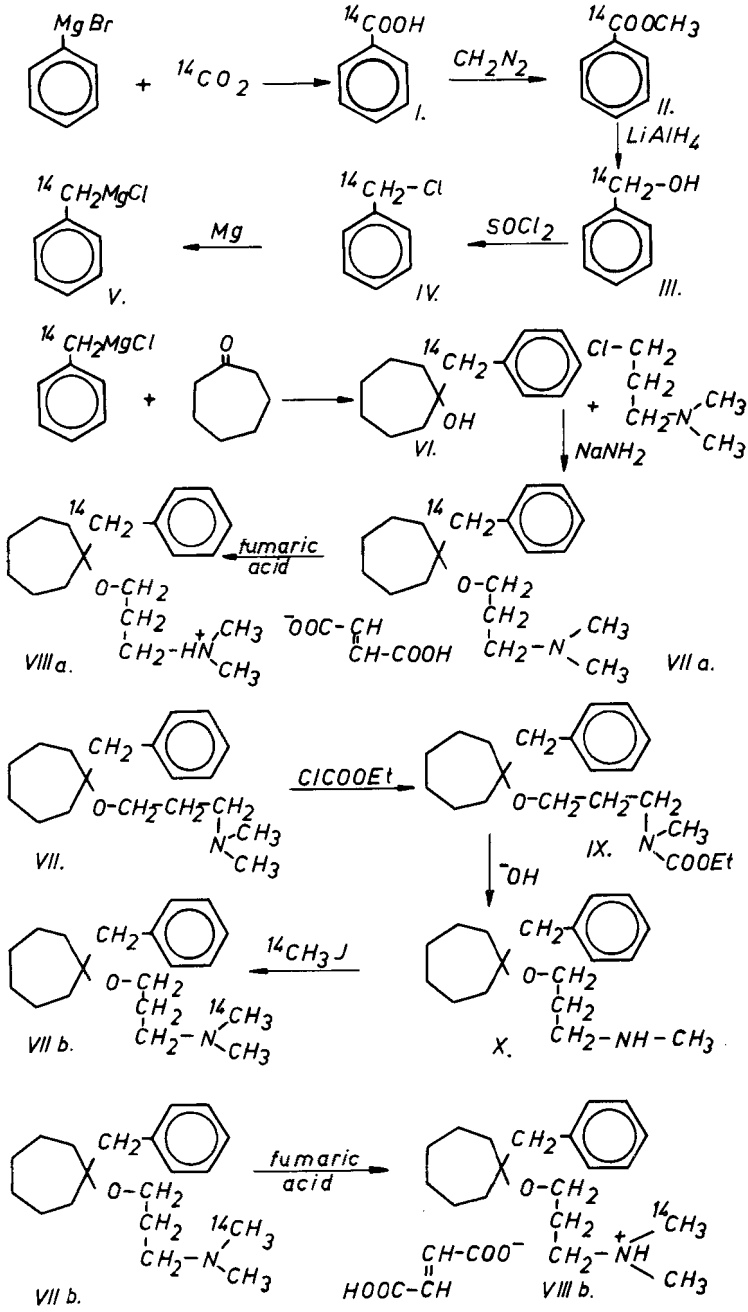
Benzoic acid-7-^{14}C (I).

Radioactive benzoic acid was synthesized as described by Calvin [5], by Grignard reaction with $^{14}CO_2$: 1.54 g of benzoic acid (12.65 mmole, 43.8 mCi/g, 5.3 mCi/mmole) having a total activity of 68 mCi was prepared.

Yield : 90 %, based on $Ba^{14}CO_3$.

Methyl benzoate-7-^{14}C (II).

1.5 g of radioactive benzoic acid (12.65 mmole) diluted with 1.75 g of inactive benzoic acid carrier to 3.25 g (27 mmole) was dissolved in 20 ml of ether and an etherael diazomethane solution was added dropwise with shaking.



VII is not labelled, VII*a* and VIII*a* have a benzyl-^{14}C label, VII*b* and VIII*b* have a methyl-^{14}C label.

Evolution of nitrogen was observed. Addition of ethereal diazomethane solution was continued until the yellow colour of the solution persisted. The reaction mixture was set aside at room temperature overnight and ether was removed at atmospheric pressure. Methyl benzoate remained as a yellowish oily product. Yield : 3.67 g (27 mmole), 100 %. The product could be used for the following operations without further purification.

Benzyl alcohol-7-^{14}C (III).

To a stirred mixture of 1.14 g (30 mmole) $LiAlH_4$ in dry ether a solution of 3.67 g (27 mmole) of methyl benzoate-7-^{14}C in 20 ml of dry ether was added dropwise in a 200 ml three-necked round bottomed flask equipped with a stirrer, dropping funnel and reflux condenser closed by a calcium chloride drying tube. The addition was performed under gentle reflux caused by the exothermal reaction. After addition the reaction mixture was heated on an oil bath under gentle reflux for 2 hours. The excess of $LiAlH_4$ was carefully decomposed by 100 ml of 20 % aqueous hydrochloric acid solution and the reaction mixture was allowed to stand overnight. The mixture separated into two phases. After taking off the ethereal phase the aqueous layer was extracted with 2 × 25 ml of ether. The combined ethereal solutions were dried over anhydrous sodium sulfate overnight, then ether was removed under atmospheric pressure to give a residual yellow oil.

Yield : 2.9 g (27 mmole), quantitative. The crude product could be used without further purification.

Benzyl chloride-7-^{14}C (IV).

A mixture of 2.9 g. of radioactive benzyl alcohol and 8 ml of freshly distilled thionyl chloride was heated under reflux on a silicon oil bath for two hours and set aside at room temperature for two days. The excess of thionyl chloride was distilled off under vacuum and the oily residue fractionated in a micro distillation apparatus. The main fraction was collected at 135-140° C/20 mm.

Yield : 2.41 g (19 mmole), 70 % based on benzyl alcohol.

1-Benzyl (7-^{14}C)-1-hydroxycycloheptane (VI).

To a stirred mixture of 0.5 g (20 mmole) of magnesium turnings and 20 ml of dry ether a solution of 1.51 g (12 mmole) of radioactive benzyl chloride in 10 ml of dry ether was added dropwise in a 100 ml three-necked, round-bottomed flask fitted with a stirrer, dropping funnel and reflux condenser closed by a calcium chloride drying tube. After addition of the first drops a vigorous reaction started with boiling of the ether. The ethereal solution was added with in 10 minutes under gentle reflux wich was regulated by an

icewater bath. After addition the solution was refluxed for two hours and then 3 ml of cycloheptanone dissolved in 10 ml of dry ether were added dropwise to the reaction mixture. During the addition the solution became slightly warm and a white, amorphous substance precipitated. After stirring for one hour a solution of ammonium chloride (5 g in 30 ml of water) was added to decompose the complex. The precipitate dissolved and the mixture separated into two phases. After separation the aqueous layer was extracted with 2 × 30 ml of ether, and the combined ethereal layers were dried over magnesium sulfate overnight, whereupon the ether was removed under atmospheric pressure. The oily residue (3.28 g) was dissolved in 10 ml of petroleum ether and kept in a cooling mixture at −50° C for half an hour. The precipitated white crystals were filtered off through a cooled filter.

Yield : 1.6 g (8.4 mmole), 70 % based on benzyl chloride.

1-Benzyl(7-^{14}C)-1-(3'-dimethylaminopropoxy)-cycloheptane (VIIa).

To a stirred mixture of 25 ml of dry benzene and 1.5 ml of 50 % sodium amide suspension in xylene a solution of 1.6 g (8.4 mmole) of 1-benzyl (7-^{14}C) -1-hydroxycycloheptane in 15 ml of dry benzene, followed by 7.5 ml of a 50 % solution of dimethylaminopropyl chloride in benzene were added dropwise in a 200 ml round-bottomed flask equipped with a stirrer, dropping funnel and reflux condenser closed by a potassium hydroxide drying tube. The mixture was refluxed in an oil bath at 110-120° C for 4-6 hours and set aside at room temperature overnight. Then 20 ml of distilled water were added dropwise stirring within 10 minutes. The yellowish emulsion obtained was separated into two distinct phases during a few hours. After separation the aqueous layer was extracted with 20 ml of benzene. After drying the benzene was distilled off under vacuum to give a yellowish oil.

Yield : 2.59 g (9 mmole), 100 %, based on VI.

1-Benzyl(7-^{14}C)-1-(3'-dimethylaminopropoxy)-cycloheptane fumarate (VIIIa).

To the solution of 2.59 g (9 mmole) of VIIa in 8 ml of ethanol a solution of 1.2 g (10 mmole) of fumaric acid in 20 ml of hot water was added. The mixture obtained was allowed to stand in a refrigerator for two days. The white crystals precipitated were filtered off with suction and washed with petroleum ether. The crude product (3.34 g) was recrystallized from a 2 : 5 mixture of ethanol and water (14 ml).

Yield : 2.96 g (7.2 mmole), 80 % based on VIIa. Radiochemical yield : 19.1 mCi (6.48 mCi/g), 41 % based on $Ba^{14}CO_3$.

1-Benzyl-1-(3'-N-methyl-N-carbethoxy)-aminopropoxycycloheptane (IX).

To a boiling solution of 1-benzyl-1-(3'-dimethylaminopropoxy)-cycloheptane (VII) (5.79 g, 20 mmole) in dry benzene (50 ml) a solution of ethyl

chloroformate (6.5 g) in dry benzene (10 ml) was added dropwise. The mixture was kept on boiling until the gas evolution ceased. Benzene was removed under reduced pressure and the residue was fractionated.

Yield : 5.2 g, 85 % based on VII.

B.p. 216°/5 mm, n_D^{20} 1.5138.

1-Benzyl-1-(3'-methylaminopropoxy)-cycloheptane (X).

The solution of 4.1 g (11 mmole) of IX, 4.7 g (84 mmole) of potassium hydroxide and 18 ml of ethanol was refluxed for 10 hours, then evaporated to dryness. The residue was dissolved in 150 ml of water. The oily layer was separated and fractionated under reduced pressure..

Yield : 3 g, 92 %.

B.p. 166°/2 mm, n_D^{20} 1.5230.

1-Benzyl-1-(3'-dimethylaminopropoxy)-cycloheptane-(N-methyl-^{14}C) (VIIb).

A 100 ml round-bottomed flask containing 40 ml of dry ether was fitted to a vacuum apparatus and frozen by liquid nitrogen. Then 22 mCi (1.419 g, 10 mmole) of radioactive methyl iodide were added into the evacuated flask from a breaking ampoule. After complete freezing of methyl iodide the system was filled with dry air at atmospheric pressure, then the reaction mixture was allowed to warm to 0° C and was treated with 4.2 g (15 mmole) of X and set aside at 0° C for 3 hours. The white, crystalline product (a mixture of a demethyl-hydroiodide of X and a quaternary salt) was filtered off and washed with 3 × 10 ml of ether. The combined ethereal solutions were evaporated under vacuum to yield 2.7 g of a yellowish oil.

This oil obtained was taken up in 50 ml of a 20 : 20 : 0.1 mixture of cycloheptane, ethyl acetate and triethylamine and filtered over a column (2.5 × 52 cm) of Brockman's Al_2O_3 (250 g). The column was eluated by the mixture of above-mentioned solvents and 10 ml fractions were collected. The desired product (VIIa) was contained in the fractions 15-30. After evaporation of these fractions under vacuum a yellowish oil remained.

Yield : 1.84 g (6 mmole), 60 % based on $^{14}CH_3I$.

1-Benzyl-1-(3'-dimethylaminopropoxy)-cycloheptane fumarate-N-methyl-^{14}C) (VIIIb).

To the solution of 1.84 g (6 mmole) of crude VIIb in 4 ml of dry ethanol a solution of 0.754 g (6.5 mmole) of fumaric acid in 15 ml of hot water was added. The solution was set aside overnight in a refrigerator, the crystals precipitated were filtered off, washed with 3 × 10 ml petroleum ether and dried over phosphorous pentoxide in a vacuum desiccator.

Yield : 1.74 g (4.2 mmole), 70 % based on VIIb. Radiochemical yield : 9.15 mCi, 5.34 mCi/g, 42 % based on $^{14}CH_3I$.

Chromatographic determinations.

Adsorbent : Kieselgel G. Solvent : dimethylformamide : triethylamine : ethanol : ethyl acetate = 5 : 5 : 30 : 60.

Sample mass : 25 μg. Detection : Dragendorff's reagent.

Autoradiogram : contact exposure on a Forte-type X-ray film for 24 hours

R_f values :	VIIb	0.65
	X	0.25
	Quaternary salt	0.00

We are indebted to Mr. T. Szarvas (Institute of Isotopes of the Hungarian Academy of Sciences) for performing the isotope analyses.

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