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SYNTHESIS OF [2-^{14}C] FANTOFARONE OR 1-[4-[3-N-METHYL N-[3,4-DIMETHYL β-PHENETHYL] AMINO PROPYLOXY] BENZENE SULFONYL]-2-ISOPROPYL [2-^{14}C] INDOLIZINE

P. POINTEAU[1,2], Y. BERGER[2]

1. C.E.A. - C.E.N. Saclay, Département de Biologie, Bâtiment 547, Service des Molécules Marquées, 91191 Gif sur Yvette Cedex, France
2. SANOFI RECHERCHE, 371 rue du Professeur J. Blayac, 34184 Montpellier Cedex 04, France

SUMMARY

Sodium [1-^{14}C] isobutyrate : 1 was obtained by carbonation of isopropyl magnesium bromide with $^{14}CO_2$. From : 1 [1-^{14}C] isobutyryl chloride : 3 was prepared, which through the successive actions of diazomethane and hydrogen bromide gave rise to 1-bromo 3 methyl [2-^{14}C] 2-butanone : 5.
The latter lead to the indolizine : 6 which by a route patterned after that used to make unlabelled Fantofarone gave the [2-^{14}C-indolizyl] Fantofarone : 8 (specific radioactivity : 58.2 mCi/mMole) overall yield 9.9 % based on [^{14}C] $BaCO_3$.

Key-words : Indolizine, carbon-14, calcium antagonist

INTRODUCTION

Fantofarone (SR 33557) is a new chemical entity synthesized by Sanofi Recherche. Preclinical pharmacology studies established its calcium antagonist properties and determined its activity as an antianginal and antihypertensive compound (1).
A synthesis of Fantofarone with carbon-14 labelling at position 3 of the side chain was recently described (2). In vitro and in vivo metabolism studies showed at least three biotransformation pathways : N-demethylation, N-dealkylation and O-dealkylation. The O-dealkylation process led us to reexamine the position of the labelled site to perform disposition studies in man and animals.

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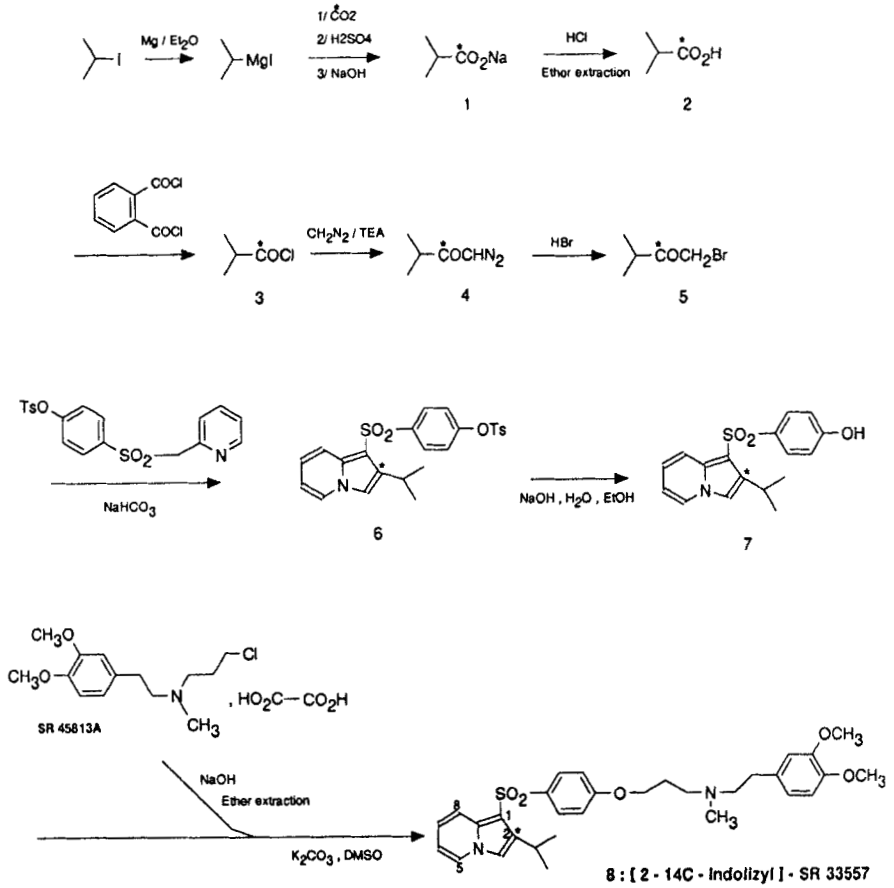
SCHEMA



(* denotes 14C label)

The present paper reports on the synthesis of Fantofarone labelled with carbon-14 at position 2 of the indolizine ring.

Incorporation of a carbon-14 atom at this position is made possible by the use of 1-bromo 3-methyl [2-^{14}C] 2-butanone : 5 in a Tschitschibabin indolizine synthesis (3,4).

DISCUSSION

The involvement of bromoketone : 5 in the classical synthesis of Fantofarone led us to regard that reagent as a possible intermediate for the insertion of an isotopic carbon into the indolizine ring.

The preparation of 1-bromo 3-methyl 2-butanone is well known and proceeds through direct bromination (5). Another classic synthetic route to monohalomethylketone is by the reaction of the corresponding diazoketone with the appropriate hydrogen halide (6, 7). [^{14}C] α-diazoketones are versatile intermediates for the synthesis of [^{14}C] compounds (8).

Since sodium salts of [1-^{14}C] carboxylic acids such as : 1 are readily available by carbonation of the homologous Grignard reagents, we choose this latter process to prepare : 5.

The transformation of : 1 into [1-^{14}C] isobutyryl chloride : 3 was performed using phthaloyl chloride (9) instead of thionyl chloride (10). Thus, the radioactive material is easily separated from the reaction mixture by vacuum-distillation. The acyl chloride : 3 is converted into 1-bromo 3-methyl [2-^{14}C] 2-butanone : 5 via the diazoketone : 4 (11, 12). Hydrogen bromide used for this transformation can be purchased, or prepared prior to use by thermolysis of triphenylphosphonium bromide (13).

The Tschitschibabin condensation was carried out by reacting the radioactive bromoketone : 5 on 4-tosyloxy phenyl 2-methyl pyridyl sulfone in presence of sodium hydrogen carbonate. Using 1.3 equivalent of : 5, the desired ^{14}C labelled indolizine : 6 was obtained in 47 % radiochemical yield after chromatographic purification.

Saponification of : 6 to give the phenol : 7 followed by a final alkylation of the phenate with the chloro compound SR 45813 afforded the expected [2-^{14}C-Indolizyl] Fantofarone : 8.

EXPERIMENTAL

General

[^{14}C] $BaCO_3$ (specific activity > 56 mCi/mmole) was obtained from C.E.A. (Saclay, France).
All chemicals used in this synthesis were of at least analytical grade. All solvents were distilled (ether from sodium/benzophenone immediately prior to use, triethylamine from CaH_2 and stored over KOH).
TLC plates, MERCK silica gel 60F254, were scanned on a BERTHOLD LB 2821 automatic TLC linear analyser.

^{14}C radioactivity was measured on a LKB Wallac 1211 Rackbetta liquid scintillation counter using PACKARD Ultima gold liquid scintillation cocktail as the scintillator.
MERCK silica gel (60, particle size 0.040 - 0.063 mm) was used for flash column chromatography.
The specific activity was determined by mass spectrometry on a Finnigan MAT Model 4600 Spectrometer.

Isopropyl magnesium iodide was prepared from 2-iodopropane according to a standard procedure (14).

[1-14C] isobutyric acid, sodium salt : 1

The apparatus used for this preparation was similar to those described in the literature (15) in use at C.E.A. for several decades (16).
Pure $^{14}CO_2$, generated by addition of concentrated sulfuric acid (large excess) on [^{14}C] barium carbonate (1.169 g - 328 mCi), was vacuum distilled into a reaction flask cooled to - 20°C containing 22 ml (22 mmoles) of a 1M ethereal solution of isopropyl magnesium iodide.
After 3 hours of reaction at room temperature under stirring, the complex was decomposed by 6N sulfuric acid (22 ml).
The reaction mixture was poured into a 500 ml round-bottomed flask containing silver sulfate (22 g) and was stirred overnight in darkness.
The ether was distilled away and [1-^{14}C] isobutyric acid was collected by steam distillation with about 250 ml of water.
The distillate was titrated with 0.1M sodium hydroxide (53 ml) to pH 8 with use of a glass electrode pHmeter to afford 1 in 80 % (262 mCi) radiochemical yield.

[1-14C] isobutyryl chloride : 3

To a solution of [1-^{14}C] isobutyric acid, sodium salt : 1 (262 mCi) in water (about 300 ml) was added 1M hydrochloric acid (10 ml).

The aqueous phase was extracted with ether (3 x 40 ml). Combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated to dryness at room temperature under 150 mm Hg to give 200 mCi of : 2 (76 %). Phthaloyl chloride (4 ml, 27.76 mmoles) was added to : 2 and the resulting mixture was refluxed with stirring under dry nitrogen for 2.5 hours. The radiolabelled acyl chloride was vacuum-distilled into a 20 ml flask with nitrogen cooling. 3 was obtained as a colourless oil and was used directly in the following step without further purification.

1-diazo 3-methyl [2-14C] 2-butanone : 4

To a stirred solution of diazomethane in ether (16) (60 ml, 0.107M solution, 6.42 mmoles) at - 78°C under dry nitrogen were added 0.90 ml of freshly distilled triethylamine (6.42 mmoles) and, over a period of 15 minutes, a solution of : 3 in anhydrous ether (4 ml).
An abondant white precipitate of triethylamine hydrochloride was rapidly formed. After one night of stirring under nitrogen at room temperature, the reaction mixture containing : 4 was used in the following step.

1-bromo 3-methyl [2-14C] 2-butanone : 5

At 0°C, dry (conc. H_2SO_4) hydrogen bromide generated according to reference (12) was allowed to bubble through the reaction mixture. 30 minutes after the end of gas evolution, water (40 ml) was added. The organic phase was separated by decanting and the aqueous phase was extracted with ether (3 x 30 ml). Combined organic phases were washed with 5 % aqueous sodium hydrogen carbonate (30 ml) and dried over anhydrous $MgSO_4$. After solvent evaporation, the ^{14}C labelled bromoketone : 5 was obtained as a pale yellow oil in 49 % radiochemical yield (98 mCi, Rf = 0.44 with ether/hexane 5:95, radiochemical purity : 95.9 %).

2-isopropyl 1-(4-tosyloxybenzene sulfonyl) [2-14C] indolizine : 6

To a solution of : 5 (54.6 mCi, 0.975 mmole) in isobutyl methyl ketone (1.5 ml) was added 4-tosyloxyphenyl 2-methypyridyl sulfone (300 mg, 0.743 mmole) followed by $NaHCO_3$ (273 mg, 3.25 mmoles).
The mixture was refluxed with stirring for 24 hours.
After cooling to room temperature, water was added and the aqueous layer was extracted twice with dichloromethane.
Combined organic phases were dried over anhydrous $MgSO_4$ and concentrated to dryness to give the crude product as an orange oil. The crude residue was purified by flash chromatography on silical gel. Unpolar impurities were eluted by ethyl acetate/hexane 10 : 90.
The expected radiolabelled indolizine was eluted using dichloromethane. Solvents were evaporated to give : 6 in 47 % radiochemical yield (25.5 mCi, Rf = 0.51 with ethyl acetate/hexane 40 : 60, radiochemical purity : 92 %).

2-isopropyl 1-(4-hydroxybenzene sulfonyl) [2-14C] indolizine : 7

A suspension of : 6 (28.9 mCi, 0.514 mmole) in ethanol (1.2 ml) and aqueous sodium hydroxide (1.2 ml, 126 g/l) was refluxed for 1.5 hours. As the reaction proceeded, the

cloudy reaction mixture turned clear. After cooling to room temperature, the mixture was diluted with water, filtered over a sintered glass and washed with dichloromethane to remove traces of starting material. At 0°C, the colourless aqueous layer was acidified to pH 1. An abondant white precipitate was formed.
After extraction with ether (3 x 20 ml), combined organic phases were dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to give : 7 in 82 % radiochemical yield (23.8 mCi, Rf = 0.37 with ethyl acetate/hexane 40 : 60, radiochemical purity : 95.3 %).

[2-14C-indolizyl] SR 33557 : 8

A solution of SR 45813 oxalate salt (246 mg, 0.68 mmole) in water (5 ml) and 35 % aqueous sodium hydroxide (200 µl) was extracted with ether (3 x 10 ml). Combined organic phases were washed twice with water, dried over anhydrous $MgSO_4$ and concentrated to dryness leaving an oily residue. The residue was taken up by 1.1 ml of dimethylsulfoxide and added to : 7 (23.8 mCi, 0.425 mmole).
Finely ground K_2CO_3 (294 mg, 2.13 mmoles) was then added. The mixture was heated at 40°C for 20 hours then diluted with water (5 ml). The aqueous phase was extracted with toluene (4 x 15 ml). Combined toluene extracts were washed twice with water, faded with activated carbon, filtered over a short bed of celite, dried over anhydrous $MgSO_4$ and concentrated. A flash chromatography on silica gel of the crude residue (21.08 mCi) using ethyl acetate/hexane/triethylamine 50:50:1 and then 75:25:1 afforded pure [2-^{14}C-indolizyl] Fantofarone as a white powder in 86 % radiochemical yield (20.5 mCi, Rf = 0.55 with toluene/tetrahydrofuran/ethanol/ammonia solution 50:50/5:1, specific activity = 58.2 mCi/mmole).

Radiochemical purity was checked by TLC (> 99.9 %) and by HPLC (99.5 %) using a ZORBAX analytical column (25 cm x 4.6 mm i.d.) and acetonitrile/water/triethylamine 75:25:0.05 as the mobile phase. The overall radiochemical yield from [^{14}C] barium carbonate was 9.9 %

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