SYNTHESIS OF [17-^{14}C] NICERGOLINE

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SUMMARY

The synthesis of 1,6-dimethyl-8β-(5-bromonicotinoyl-[^{14}C]oxymethyl)-10α-methoxy-ergoline ([17-^{14}C] nicergoline) is reported. A five-step route starting from the addition of potassium [^{14}C] cyanide **2** to 1,6-dimethyl-8β-chloro-10α-methoxy-ergoline **1** yielded the expected [17-^{14}C] nicergoline, 97% radiochemically pure, with a specific activity of 2.23 GBq/mmol. The overall radiochemical yield was about 10% from **2**.

Key words: Ergoline, Nicergoline, [17-^{14}C] Nicergoline, Sermion, Senile dementia.

INTRODUCTION

Nicergoline, 1,6-dimethyl-8β-(5-bromonicotinoyl-oxymethyl)-10α-methoxy-ergoline, is an ergot derivative synthesised by Farmitalia (Milan) in the mid-1960s [1]. It was initially thought to act as a vasodilator (it was registered in Italy in 1972 under trademark Sermion®), proven by various animal and man studies [2-3]. More recent research has outlined the actions of nicergoline on the central nervous system and accounted for its positive effect on cognitive function [4-6].

Nicergoline is well tolerated and is currently used in the treatment of senile dementia of the Alzheimer's type (SDAT) and multi-infarct dementia (MID) [7]. The first ADME studies in animals and man [8] were performed with nicergoline labelled with tritium either randomly or in position 17, and with doubly labelled [^{3}H,^{14}C] nicergoline [9]. The results obtained from these studies indicated the importance of the labelling with carbon-14 in the ergoline moiety to better evaluate the fate of this drug by minimizing the

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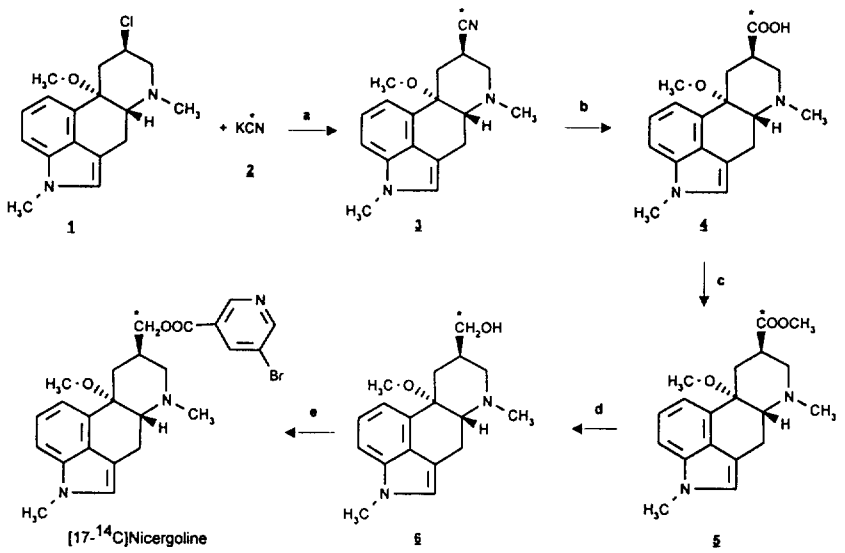
biases produced by tritium exchange. Previous studies carried out with ergoline derivatives [10-11], indicated the carbonyl group attached to the position 8 as a possible and convenient labelling site. In fact this radiolabel can reasonably be expected to remain present in the possible nicergoline metabolites containing the ergoline skeleton [8].

RESULTS AND DISCUSSION

[17-^{14}C] Nicergoline was obtained according to the procedure depicted in the Scheme.

SCHEME



* = ^{14}C
Reagents and conditions
a) - Ethanol:water, reflux. b) - 10N NaOH in ethanol, reflux. c) - H_2SO_4 in methanol, 0°C.
d) - $LiAlH_4$ in THF, reflux. e) - 5-Bromonicotinic acid chloride, imidazole in methylene chloride, 40°C.

Commercially available potassium [^{14} C] cyanide 2 was added to 1,6-dimethyl-8β-chloro-10α-methoxy-ergoline 1 in ethanol:water at reflux, as described for cabergoline, a similar compound [12], to give the crude compound 3, which was purified by flash-chromatography. The purified nitrile derivative 3 was then submitted to alkaline hydrolysis, according to a method described for other ergoline compounds [10], to give the carboxylic acid 4. The conversion of 4 to the corresponding methyl ester 5, was obtained by reaction with methanol in the presence of H_2SO_4. The subsequent reduction with lithium aluminum hydride in tetrahydrofuran yielded the 8β-hydroxymethyl derivative 6. The final acylation was carried out by addition of 6 to an excess of freshly prepared 5-bromonicotinic acid chloride at 40° in anhydrous

methylene chloride in the presence of imidazole. This last reaction was not carried out as described in Vicario et al. [9] because of the low and not reproducible yield. The substitution of pyridine with imidazole in methylene chloride according to the internal procedure [13] allowed a good yield of the final compound to be obtained even when scaling down to 50 μmoles. The purification of the resulting crude material by preparative TLC gave the expected [17-^{14}C] nicergoline with a radiochemical purity >97% and a specific activity of 2.23 GBq/mmol. The overall radiochemical yield was about 10% from 2.

EXPERIMENTAL

General methods

Potassium [^{14} C] cyanide was purchased from Amersham International p.l.c..All solvents and reagents were of analytical grade and were used without purification unless otherwise indicated. The intermediate 1 and the authentic unlabelled samples used to identify the labelled material, were supplied by Dr. Mantegani (Medicinal Chemistry Department, R&D CNS, Pharmacia & Upjohn, Nerviano, Italy).

Radioactivity measurements were carried out with a Packard 300C liquid scintillation counter using Rialuma (Lumac System A.G.) as liquid scintillation cocktail.

Ultraviolet spectra were determined on a Beckman DU 50 UV/VIS spectrophotometer. Radiochemical analyses of thin layer chromatography (TLC) plates were performed with a Packard Bioscan System 200 imaging scanner. High performance liquid chromatography (HPLC) analyses were carried out at ambient temperature with a Perkin-Elmer pump series 410 equipped with a Perkin-Elmer LC 295 UV/VIS spectrophotometer (λ =280 nm). Radiochemical purities were determined by HPLC using a Packard Trace II model 7150 radioactivity flow detector. All labelled materials were identified by chromatographic comparison with the corresponding authentic unlabelled samples using the following chromatographic methods:

Thin layer chromatography (TLC)

TLC was carried out, where not specified, using Merck silica gel F254 plates, 20x5 cm, 0.25 mm thick. The eluting solvent systems were as follows with proportions by volume:

A -	ethyl acetate:benzene:petroleum ether (bp 40-60°C)	70:25:5	(by volume)
B -	chloroform:methanol	4:1	(by volume)
C-	ethyl acetate:cyclohexane:methanol	4:2:1	(by volume)
D-	chloroform:methanol:glacial acetic acid:water	80:20:14:6	(by volume)
E-	hexane:ethyl acetate:diethylamine	5:5:1	(by volume)

F-	cyclohexane:acetone:diethylamine	7:2:1	(by volume)
G-	benzene:acetone:diethylamine	7:2:1	(by volume)
H-	hexane:chloroform:ethyl acetate:diethylamine	5:5:10:1	(by volume)
I -	chloroform:methanol	9:1	(by volume)

High performance liquid chromatography (HPLC)

Analyses were performed at room temperature, using a Macherey-Nagel Nucleosil C8 column, (250x4.6 mm I.D.; particle size 10 μm) eluted with acetonitrile : 0.01M K_2HPO_4 buffer, pH6, containing 1% triethylamine (58:42 by volume) at 1.6 ml/min.

1,6-Dimethyl-8β-[^{14}C]cyano-10α-methoxy-ergoline (3)

Three vials containing potassium [^{14}C] cyanide 2 (0.340 GBqx3 = 1.11 GBq; 0.514 mmol) were placed in a glove box and carefully opened. The radiolabelled compound was then dissolved with ethanol (5x3 =15 ml) and water (0.5x3 = 1.5 ml) and the resulting solution was added to 1,6-dimethyl-8β-chloro-10α-methoxy-ergoline 1 (156.26 mg; 0.514 mmol). The reaction flask was stoppered with a teflon stopper and transferred to a fume cupboard. After removing the stopper, the reaction flask was equipped with a reflux condenser connected to two traps in series filled with 1N NaOH in order to collect any radioactive volatile compound eventually produced. The reaction mixture was then stirred at reflux for three hours. At the end of the reaction (determined by radio-TLC; system A), the solvent was removed in vacuum using a rotating evaporator connected to a dry-ice condenser equipped with a flask filled with 1N NaOH as a safety precaution (see above). The residue was taken up with ethyl acetate (~50 ml) and water (~10 ml) and transferred into a separating funnel. After separation, the organic layer was washed with saturated aqueous $NaHCO_3$ (~7 ml), dried (Na_2SO_4) and evaporated to give the crude intermediate 3 (984 MBq) 68% radiochemically pure (by radio-TLC; system A). This intermediate was purified by flash chromatography (column: 45x3.5 cm I.D.; Silica gel Merck 60; 230-400 mesh ASTM: 50 g). The column was eluted with a mixture (100 ml) of acetone:cyclohexane 1:9 (by volume), then with acetone:cyclohexane 2:8 (by volume) collecting 2 fractions (50 ml each) and 25 fractions (10 ml each). Fractions 19 to 24 were combined to yield, after solvent evaporation to dryness, compound 3 (584.86 MBq), 98% radiochemically pure (by radio-TLC ; system A: Rf = 0.18). The radiochemical yield was 52% from 2.

1,6-Dimethyl-8β-[^{14}C]carboxy-10α-methoxy-ergoline (4)

The intermediate 3 (195 MBq; 0.090 mmol) was dissolved in ethanol (2 ml) and evaporated to dryness. This operation was repeated twice and, finally, the residue was dried under vacuum for 1 hour. After

addition of ethanol (3.35 ml) and 10N NaOH (2.34 ml), the reaction mixture was stirred at reflux overnight. At the end of the reaction (determined by radio-TLC; system B), the solution was cooled to 0°C (ice bath), adjusted to pH 10 with 37% HCl and evaporated to dryness. The solid was dissolved in toluene (~3 ml) and evaporated to dryness. This operation was repeated three times with toluene and three times with methanol. After drying under vacuum for 30 minutes, the intermediate 4 was recovered, 94% radiochemically pure (by radio-TLC ; system B: Rf = 0.16). The radioactive compound was used without further purification in the next step.

1,6-Dimethyl-8β-[^{14}C]carbomethoxy-10α-methoxy-ergoline (5)

A cold (0-5°C) solution of 96% H_2SO_4 (1.17 ml) in methanol (3 ml) was added dropwise to a cooled (0°C) suspension of intermediate 4 (195 MBq; 0.09 mmol) in methanol (2.33 ml). The reaction mixture was stirred at room temperature for 2 hours then poured into a 50 ml round-bottom flask filled with crushed ice, then adjusted to pH 8 with 30% NH_4OH. The aqueous solution was transferred to a separating funnel and extracted with ethyl acetate until a colourless organic phase was obtained. The organic extracts were combined, washed with brine to neutrality, dried (Na_2SO_4) and evaporated to yield the intermediate 5, 81.6% radiochemically pure (by radio-TLC; system C: Rf = 0.38), that was used without further purification in the next step.

1,6-Dimethyl-8β-[^{14}C]hydroxymethyl-10α-methoxy-ergoline (6)

The intermediate 5 (195 MBq; 0.090 mmol) was dissolved in THF (3 ml) and evaporated to dryness. This operation was repeated twice and, finally, the residue was dried under vacuum for 2 hours. Lithium aluminum hydride (28.2 mg; 0.74 mmol) was carefully added to a solution of 5 (195 MBq; 0.09 mmol) in THF (1.95 ml). Then the suspension was refluxed under stirring for 60 minutes. At the end of the reaction (determined by radio-TLC; system D), the mixture was cooled to 0°C and the excess of reagent was decomposed by slow addition of 10% water in THF (4 ml). After stirring at room temperature for 30 minutes, the solvent was evaporated to dryness. The residue was taken up with water (20 ml), transferred to a separating funnel and extracted with chloroform until a colourless organic phase was obtained. The organic phase was dried (Na_2SO_4) and, after solvent evaporation to dryness, gave the intermediate 6 (147.6 MBq; 0.068 mmol), 80.5% radiochemically pure (by radio-TLC; system D: Rf = 0.5), that was used without further purification in the next step.

The radiochemical yield was 75% from 3 .

1,6-dimethyl-8β-(5-bromonicotinoyl-[^{14}C]oxymethyl)-10α-methoxy-ergoline ([17-^{14}C]nicergoline)

Imidazole (46.8 mg; 0.687 mmol) in methylene chloride (0.4 ml) was added with 5-bromonicotinic acid (30 mg; 0.148 mmol). The solution was cooled to 0°C and thionyl chloride (13.7 ml; 0.188 mmol) in methylene chloride (0.137 ml) was slowly added. After stirring at reflux for 2 hours, the reaction mixture was evaporated to dryness, dissolved with toluene (~3 ml) and evaporated to dryness. This operation was repeated twice with methylene chloride. Then, the residue was dried under vacuum for 45 minutes. The solid was suspended in methylene chloride (0.4 ml) and, after addition of intermediate 6 (147.63 MBq; 0.068 mmol) in methylene chloride (1.3 ml), the reaction mixture was refluxed under stirring for 3 hours. At the end of the reaction (determined by radio-TLC; system I), the mixture was adjusted to pH 8 with 0.1N NaOH, methylene chloride (~5 ml) was added and then it was transferred to a separating funnel. After separation of the aqueous solution, the organic phase was washed with water to neutrality and dried (Na_2SO_4). Evaporation of the solvent to dryness, yielded crude [17-^{14}C]nicergoline (108.67 MBq), 60.7% radiochemically pure (by radio-TLC; system I). The above crude material was purified by preparative-TLC (Merck F254 plate, 20x20 cm, 1 mm thick) using system H as eluting solvent system. The chromatographic band corresponding to the compound was removed and the product extracted from silica gel with acetone (~30 ml). The organic extracts were combined and, after solvent evaporation to dryness, [17-^{14}C] nicergoline (43.10 MBq), 94% radiochemically pure (by radio-TLC; system E; system H; system I) was obtained. The radiolabelled compound was purified again by preparative-TLC using the same procedure. After solvent evaporation to dryness, [17-^{14}C] nicergoline (36.50 MBq; 0.016 mmol) was obtained with a radiochemical purity >97% [by radio-HPLC Retention Time (RT) = 11min; by radio-TLC; system E: Rf=0.37 ; system F: Rf=0.36 ; system G: Rf=0.63 ; system H: Rf=0.60 ; system I: Rf=0.57].

The overall radiochemical yield was about 10% from 2.

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