NOTE

Synthesis of [pyrrolidine-^{14}C]-cotinine from ^{14}C-nicotine

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Summary

The synthesis and purification of ^{14}C-cotinine is reported. ^{14}C-nicotine is converted into ^{14}C-dibromocotinine. Debromination of the latter results in ^{14}C-cotinine which is purified by high performance liquid chromatography.
The total radioactive yield of the synthesis is 44.8 %.
Starting from 500 μCi ^{14}C-nicotine, 224 μCi ^{14}C-cotinine is obtained with a specific activity of 0,23 mCi/mmol.

Key Words

^{14}C-cotinine, [pyrrolidinone-5-^{14}C]-1-methyl-5-(3-pyridinyl)-2-pyrrolidinone, ^{14}C-nicotine, [pyrrolidine-2-^{14}C]-3-(1-methyl-2-pyrrolidinyl)pyridine, ^{14}C-dibromocotinine.

Introduction

The current interest in cotinine is due to the fact that cotinine is an appropriate and specific biological marker for the smoking behaviour. Cotinine is a major metabolite of nicotine in human and in a lot of mammals (1), and its biological half-life is considerably longer than for nicotine (2,3,4). The method of Morselli et al. (5), described

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for the synthesis of cotinine with labelling in the pyrrolidinone part of the molecule is rather long and of a low reported total yield. Since [^{14}C-pyrrolidine]-nicotine is now commercially available, the method of Bowman and McKennis for unlabelled cotinine (6) was adapted to the specific needs of the radioactive synthesis. To obtain ^{14}C-cotinine of great purity, the final product was purified by HPLC.

Experimentals

a. general

Radiolabelled nicotine [pyrrolidine-2-^{14}C]-3-(1-methyl-2-pyrrolidinyl)pyridine, (2 x 250 µCi in 2.5 ml ethanol), with a specific activity of 50.5 mCi/mmol and a radiochemical purity higher than 97 % was purchased from New England Nuclear (NEC-689). (-)Nicotine was purchased from Fluka and purified by a vacuum distillation prior to use.

All other reagents for the synthesis were of technical grade, except for chloroform, anhydrous sodium sulphate and 25 % ammonia solution (analytical quality, Merck).

High Performance Liquid Chromatography (HPLC) was used for the purification of the labelled cotinine (Waters Associates, M-6000 A) equipped with an UV detector (Waters Associates, Model 440) and a Waters U6K Injector. The preparative column (250 x 10 mm) was filled with 10 µm silica gel. The mobile phase consisted of chloroform/hexane/methanol/25 % ammonia solution : 1000/875/115/10. For analytical control, a Lichrosorb column (Si 60, 7 µm, 250 x 4 mm) and the same solvent system was used.

Radiochemical purity was determined by thin layer chro-

matography (TLC) using 0.25 mm silica gel pre-coated TLC-plates Sil G-25 UV 254 (Machery-Nagel). Radio thin layer chromatograms were examined by a thin layer scanner II LB 2723 (Berthold).

Quantification of cotinine was done by UV spectrometry on a SP8-400 UV/VIS spectrophotometer (Pye Unicam) at 263 nm.

Radioactive samples were counted in Lumagel (Lumac) using a Liquid Scintillation beta-Counter (Berthold BF 5003 A). Counting efficiency was established by adding ^{14}C toluene standard with a specific activity of 4.5×10^5 d.p.m./ml (New England Nuclear NES-006) and recounting the sample.

Mass spectra were recorded and chemical purity examined on a HP 5995 A GC-MS System consisting of a quadrupole spectrometer, a Model 5710A gas chromatograph, an HP 1896 A capillary injector an an HP 9825B data system. A capillary column (CP-Sil 5 CB, 25 m x 0.32 mm i.d.) was used. The mass spectrometer was operated with an electron-ionisation energy of 70 eV and a source temperature of 148°C. The source pressure was 10^{-5} Torr and the accelerating voltage 1.6 kV. The temperature of the GC-MS transfer line was set at 280°C. A fused-silica chemically bounded CP-Sil 5 CB, 25 m x 0.32 mm i.d. column was used. The temperature was initially kept at 100°C for 2 min. then raised to 270°C at a rate of 5°C/ min and held for another 10 min. The retention time of cotinine was 9.86 min.

b) Preparation of ^{14}C-dibromocotinine (2)

To radiolabelled nicotine[pyrrolidine-2-^{14}C] (1) (1.6 mg in 5.0 ml ethanol; 0.01 mmol; 50.5 mCi/mmol), unlabelled

distilled nicotine (338 mg; 2.08 mmol) is added followed by 1 ml of 50 % w/v tartaric acid (3.33 mmol) in water. After mixing, ethanol is evaporated on a water bath (45°C) under a gentle stream of nitrogen. The residue is suspended in 1.5 ml 80 % v/v aqueous acetic acid. Under constant stirring 2.2 ml of a solution of bromine (0.8 ml; 15.6 mmol) in 80 % v/v aqueous acetic acid is introduced dropwise. The addition is completed in a period of 2 hours. Temperature is kept below 47°C during that period. The mixture is stirred for another 2 hours. Water (4 ml) is added and the mixture heated to 80°C, until a homogeneous solution is obtained. The solution is then cooled slowly to room temperature and kept in a refrigerator (4°C) overnight. A yellow-reddish precipitate of the hydrobromide perbromide of dibromocotinine is separated by filtration over a glass-filter. The precipitate is washed abundantly with water and dried at room temperature.

Yield : 923.0 mg (77 %)
Radiochemical purity : > 97 % (R_f = 0.74; solvent system: toluene/acetone/methanol/25% ammonia solution : 50/45/10/5).

Mass spectrum m/z (rel. intensity) : 42.1 (40.1), 78.1 (17.1), 96.1 (12.8), 98.1 (14.1), 117.0 (100.0), 253.1 (7.4), 254.0 (14.8), 255.0 (8.2), 277.0 (15.3), 332.0 (3.5), 334.0 (7.1), 336.0 (3.2)

c) Preparation and purification of ^{14}C-cotinine (3)

The precipitate of ^{14}C-dibromocotinine hydrobromide perbromide is suspended in a mixture of 2.5 ml 50 % v/v aqueous acetic acid and 0.3 ml concentrated hydrochloric

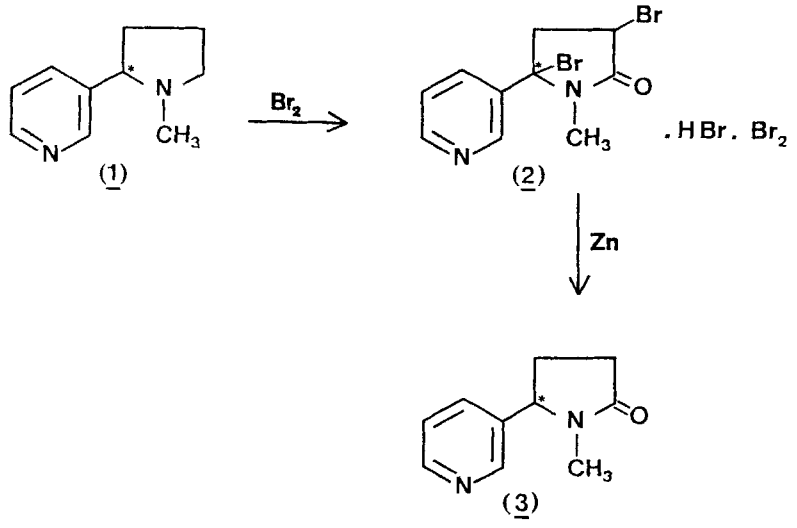
acid. Under constant stirring, 1 g zinc powder is added in small portions in a period of 2 hours. The mixture is then stirred for another 2 hours and the excess of zinc removed by filtration. The filter is washed with water. The pH of the combined filtrate and wash liquors is adjusted to 8.5 with 25 % ammonia solution. The mixture is extracted three times with chloroform. The combined organic extracts are evaporated until a yellowbrown oil is obtained. The latter is dissolved in 1.0 ml mobile phase and the total volume injected on a preparative silica gel column. Different fractions were collected and analysed by HPLC. Fractions containing pure cotinine are pooled and the solvent removed under reduced pressure. Fractions, containing also impurities, were pooled separately, concentrated under reduced pressure and chromatographed again. After three runs, most of the cotinine is purified and recovered.

Yield : 172 mg (60.7 %)
Radiochemical purity : > 99.5 % (R_f = 0.59; solvent system : toluene/acetone/methanol/25% ammonia solution : 50/45/10/5).
Mass spectrum m/z (rel. intensity) : 39.1 (12.9), 41.1 (19.0), 42.1 (31.9), 51.1 (13.7), 69.1 (6.0), 78.1 (6.5), 98.1 (100.0), 99.1 (5.2), 118.1 (12.8), 119.1 (13.0), 121.2 (6.4), 147.2 (6.1), 176.2 (25.6)
Specific activity : 0.23 mCi/mmol

Results and discussion

Different other methods have been described to prepare cotinine, including a total synthesis (7,8,9). Since labelled nicotine is now commercially available, it showed more interesting to synthesise cotinine using a shorter

pathway. The method of Bowman and McKennis, previously described for the synthesis of unlabelled cotinine, was therefore adapted to the specific needs of this radioactive synthesis. The reaction scheme is as follows :



(*) : position of radiolabelled carbon

A first reaction step involves oxidation with bromine, however simultaneously coupled with a substitution of bromine in the molecule. This method allows an easy isolation of the intermediate by filtration. In the second reaction step, the intermediate is reduced by zinc and the excess of reagent is again easily removed by filtration. Some particular problems had to be solved for the synthesis of ^{14}C-cotinine. First, the synthesis had to be carried out starting from very small amounts of radioactive nicotine (0.01 mmol, 1.6 mg). Since ^{14}C-cotinine was intended for whole-body autoradiography in the Marmoset monkey (Callitrix jacchus) and cotinine toxicity is much lower than that of nicotine, a high specific activity was not required. By

diluting labelled nicotine with unlabelled, an acceptable yield of ^{14}C-cotinine was obtained with still a sufficient radioactivity to allow autoradiography.

The ethanol in which the labelled nicotine was dissolved had to be removed previously, since the dibromocotinine does not precipitate in an alcoholic medium. In order to avoid losses of nicotine during evaporation, nicotine was first converted into its tartrate salt. Finally, the amount of synthesised crude product was too small to allow a vacuum distillation (6). HPLC showed to be the most appropriate method for purification. The total yield of the synthesis, starting from 500 μCi nicotine-[pyrrolidine-2-^{14}C] was 44.8 % (224 μCi).

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