# A CONVENIENT SYNTHESIS OF $^{14}$C-COTININE FROM $^{14}$C-NICOTINE.

Dhimant H. Desai, Mirjana V. Djordjevic, Shantu Amin
Naylor Dana Institute for Disease Prevention
American Health Foundation, Valhalla, New York 10595.

## SUMMARY

A convenient synthesis with analytical monitoring of $^{14}$C-cotinine is reported. $^{14}$C-Nicotine was converted into $^{14}$C-dibromocotinine hydrobromide perbromide. Debromination, achieved by using Zn dust/acetic acid, resulted in high yields (71%) of $^{14}$C-cotinine.

KEY WORDS: $^{14}$C-cotinine, (pyrrolidone-5-$^{14}$C)-1-methyl-5-(3-pyridyl)-2-pyrrolidone. $^{14}$C-nicotine, (pyrrolidine-2-$^{14}$C)-3-(1-methyl-2-pyrrolidinyl)pyridine. $^{14}$C-bromocotinine.

## INTRODUCTION

The major metabolic pathway of nicotine proceeds via oxidation of the pyrrolidine ring. Cotinine, the principal metabolite of nicotine, is then further metabolized by α-hydroxylation to trans-3'-hydroxycotinine or by hydrolysis to 4-(methylamino)-4-(3-pyridyl)butyric acid (1,2).

While cotinine syntheses have been known for a long time, very little is known about efficient $^{14}$C-cotinine synthesis. The method described by Morselli et.al. (3) is time consuming and affords only a low yield. Recently, Peeters and Daenens (4) synthesized $^{14}$C-cotinine by adopting the method developed for unlabelled cotinine by Bowman and McKennis (5). This led to $^{14}$C-cotinine with a specific activity of 0.23 mCi/mmol (8.5 MBq/mmol) in 44.8% yield.

For our study on plant metabolism, $^{14}$C-cotinine of higher specific acitivity was required. We now report a convenient synthesis of $^{14}$C-cotinine with higher specific activity, based on the method of Bowman and McKennis (5). The cotinine obtained was >97% pure according to HPLC analysis.

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## EXPERIMENTAL

Racemic R,S-(±)-nicotine [(2-$^{14}$C)pyrrolidine] **(1)** stored in ethanol (0.16 mg/500 μL) under argon was obtained from New England Nuclear (Boston, MA). Its specific activity was 50.0 mCi/mmol (1.85 GBq/mmol). Radiochemical purity (>97%) was established by HPLC using a Zorbax ODS reverse phase column (DuPont Company, Wilmington, DE) (4.6 mm x 250 mm, 5 μm), and 1% triethylamine acetate in $H_2O$ at pH 4:acetonitrile (65:35) as mobile phase.

(-)Nicotine, purchased from Aldrich Chemical Co. (Milwaukee, WI), was purified by vacuum distillation prior to use. All other reagents and solvents were also purchased from Aldrich Chemical Co., but were used without further purification.

All analyses were performed on a Waters (Bedford, MA) liquid chromatographic system comprised of an automated gradient controller and a Zorbax ODS reverse phase column (4.6 mm x 250 mm). Absorbance at 254 nm was monitored on a UV/Vis detector (Model M116, Gilson Electronics, Middleton, WI). We used a binary gradient mobile phase consisting of solvent systems A and B. Solvent A was 1% (v/v) triethylamine acetate in $H_2O$ at pH 4, solvent B was acetonitrile.

The solvent program began with an initial 10-minute run of 15% A:85% B, at a flow rate of 1 mL/min. Then, the solvent ratio was changed to 30% A:70% B and was run at 1 mL/min for 50 minutes. Retention times of $^{14}$C-cotinine and $^{14}$C-nicotine are 4-6 min and 18-19 min respectively.

The HPLC effluent was monitored with a Radiomatic FLO-ONE/Beta radioactivity flow detector system (Radiomatic Instruments, Tampa, FL).

Counting efficiencies were established by comparing dpm values of respective fractions as collected. DPM were measured using a monoflor scintillation fluid (National Diagnostic, Manville, NJ) in a Beckman LS9800 scintillation counter.

Cotinine was quantified by UV spectrometry on a Hewlett-Packard 8452 diode array spectrophotometer using the Hewlett-Packard 89530 MS-DOS UV/Vis program in methanol. The extinction coefficient for cotinine was $\lambda_{max}$ (MeOH) 255 nm ($\epsilon$ 3244), 262 nm ($\epsilon$3590), 268 nm ($\epsilon$ 2600).

The yield and purity of cotinine in the reaction with cold nicotine were monitored on a Hewlett-Packard Model 5890 gas chromatograph equipped with a splitless injector and a flame ionization detector (FID), which was interfaced with a Hewlett-Packard Model 3390A integrator (Hewlett-Packard, Paramus, NJ). The reaction products were separated on a 30-m DB-5 fused silica capillary column (0.25 mm i.d.; 0.25 $\mu$m film thickness). Injector and detector temperatures were maintained at 230 and 260°C, respectively. The oven temperature was programmed with an initial 3-min hold from 60 to 180°C, at 25°C/min, it was then held at 180°C for 3 min before being raised to 230°C at 2°C/min; it was finally held at 230°C for 12 min. Under these conditions the retention times for nicotine, N-nitrosoguvacoline (an internal standard used for the quantitation of reaction products) and cotinine were 9.23, 11.51 and 14.96 min, respectively.

Preparation of $^{14}$C-bromocotinine hydrobromide perbromide **(2)**:

Radiolabelled nicotine (pyrrolidine-2-$^{14}$C) **(1)** (0.16 mg, in 500 $\mu$L EtOH (1.85 MBq/500 $\mu$L), 0.001 mmol) and unlabelled distilled nicotine (0.78 mg, 0.0048 mmol) were mixed in 500 $\mu$L of 80% v/v aqueous acetic acid in a 2 mL screw cap reaction vial. With stirring, 500 $\mu$L of a solution of bromine (35 $\mu$L, 108.6 mg, 0.68 mmol) in 80% v/v aqueous acetic acid was introduced slowly. The mixture was then heated in a water bath at 40-45° for 2h in a closed reaction vial.

The reaction mixture was then evaporated to dryness in a slow stream of nitrogen to yield yellow-reddish crude dibromocotinine hydrobromide perbromide, which was used in the next step without further purification.

Preparation of $^{14}$C-cotinine (3):

The dibromocotinine hydrobromide perbromide was dissolved in a mixture of 500 µL of 50% v/v aqueous acetic acid and 20 µL concentrated hydrochloric acid. With stirring, 5 mg zinc powder was added within 2 min. The mixture was then stirred for 1h, another portion of zinc powder (5 mg) was added, and stirring was continued for another hour. The excess zinc powder was then removed by filtration, t5he zinc powder washed with water, and the pH of the filtrate adjusted to 9.5 with 5N ammonia. The mixture was then extracted with chloroform (6 x 5 mL) and the chloroform evaporated. The dry product was dissolved in 1 mL MeOH for HPLC, UV, and GC analyses.

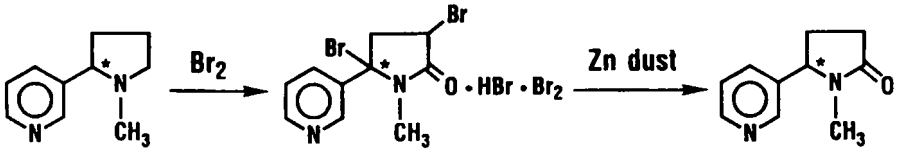
Yield: 0.78 mg (71%).

Radiochemical purity: >97 %.

Specific activity: 8 mCi/mmol (296 MBq/mmol).

## RESULTS AND DISCUSSION

The synthesis of unlabelled cotinine has been reported in the literature (6,7,8). Labelled nicotine is now commercially available, thus facilitating synthesis of cotinine with the modified procedure of Bowman and McKennis (5). The reaction sequence is as shown:




The first reaction step involves oxidation of nicotine with bromine. In the second step, the dibromocotinine intermediate is reduced by an excess of zinc dust and acetic acid.

To obtain $^{14}$C-cotinine with a higher specific activity, it was necessary to modify the procedure reported by Peeters and Daenens (4). We have eliminated the formation of nicotine tartrate to

avoid loss of nicotine during the evaporation of EtOH. We have also abstained from evaporating the EtOH in which the labelled $^{14}$C-nicotine was supplied. By using a large excess of bromine to convert $^{14}$C-nicotine into $^{14}$C-dibromocotinine and reducing it with Zn dust, we obtained a fairly high yield of $^{14}$C-cotinine (71%).

The product was of high purity (>97%) and thus required no further purification. The specific activity of the $^{14}$C-cotinine was 8 mCi/mmol (296 MBq/mmol).

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