SYNTHESIS OF [METHYL-^{14}C]-N-METHYLPUTRESCINE

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SUMMARY

[Methyl-^{14}C]-N-methylputrescine was prepared from [^{14}C]methylamine hydrochloride in five steps. Reaction of benzaldehyde and [^{14}C]methylamine (10 mCi) followed by catalytic hydrogenation yielded [methyl-^{14}C]-N-methylbenzylamine. The key step in this process is the alkylation of [methyl-^{14}C]-N-methylbenzylamine in aqueous medium with 4-bromobutyronitrile. The radiochemical purity of the final product after two successive catalytic hydrogenations was in excess of 97%. The radiochemical yields in two successive runs were 26 and 38%, based on starting [^{14}C]methylamine, with specific activities of 22 and 23 mCi/mmol, respectively. This sequence provides a convenient and efficient regioselective radiosynthesis of [methyl-^{14}C]-N-methylputrescine.

KEYWORDS: ^{14}C-methylamine hydrochloride, [methyl-^{14}C]-N-methylputrescine, carbon-14.

INTRODUCTION

N-Methylputrescine (**1**), a key intermediate in the biosynthesis of nicotine and the tropane alkaloids (1), is formed in tobacco plants primarily in the roots from putrescine (**2**) (2). As part of an ongoing tobacco research program, we required radiolabelled N-methylputrescine with radiochemical purity in excess of 97%.

$CH_3NHCH_2CH_2CH_2CH_2NH_2$

1

$H_2NCH_2CH_2CH_2CH_2NH_2$

2

A number of syntheses of N-methylputrescine have been described (3). The symmetrical nature of putrescine (**2**) renders it of limited value as a starting material. One large scale synthesis of **1**, however, has been described (4) and involves formylation of putrescine followed by chromatographic separation of the formylated products. Frydman, et al. (3), developed an

CCC 0362–4803/94/050421–06

Received 1 October, 1993
Revised 10 December, 1993

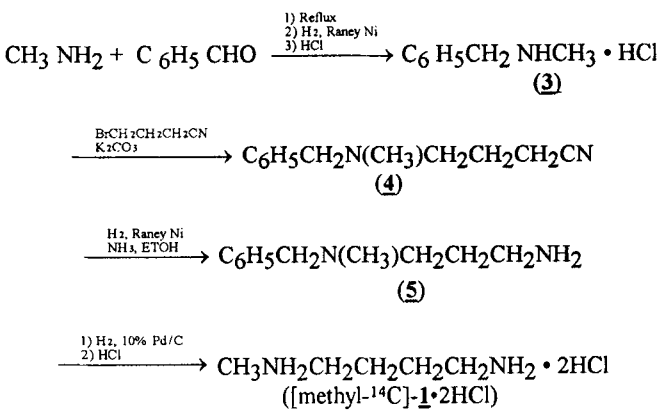
alternative approach for the preparative synthesis of N-methylputrescine and its homologues, involving the condensation of alkylbenzylamines with 4-bromobutyronitrile. A mixture of products was obtained when methylamine was reacted with 4-bromobutyronitrile. The synthesis of labelled N-methylputrescine was reported by Leete and McDonell (1) from labelled potassium cyanide and ethyl 4-bromobutanoate but it was necessary to remove potassium hydroxide as a contaminant in the labelled potassium cyanide for the reaction to proceed in reasonable yield.

In this report we describe the radiosynthesis of [methyl-^{14}C]-N-methylputrescine using [^{14}C]methylamine as the starting material and a modified synthesis based on the work of Frydman, et al (3). The procedures employed were based on consideration of various factors: availability and purity of labelled material, amenability of reactions to small scale synthesis, purification of intermediates and expected yield.

RESULTS AND DISCUSSION

The reaction sequence was thoroughly investigated with unlabelled materials and the identity of the products confirmed prior to using any labelled material. [^{14}C]Methylamine was released from its hydrochloride salt by treatment with potassium hydroxide followed by distillation. The distillate was collected in a receiver containing benzaldehyde and the mixture was then refluxed to form the Schiff base. Catalytic reduction of the Schiff base yielded [methyl-^{14}C]-N-methylbenzylamine (**3**) (see Scheme). This procedure was found to be effective for essentially quantitative conversion of millimolar amounts of methylamine hydrochloride to the imine and subsequent reaction to **3**. Thin layer radiochromatography (TLRC) of crude **3** showed 97% of the net activity to be associated with the desired product which was converted to its hydrochloride salt and used in the next step without further purification.

Scheme



In the synthesis of N-methylputrescine reported by Frydman, et al. (3), the alkylation of N-methylbenzylamine was performed in non-aqueous medium (1-butanol) with anhydrous potassium carbonate. We investigated the reaction in aqueous medium to facilitate the handling of radiolabelled N-methylbenzylamine as its hydrochloride salt. The reaction proceeded smoothly to yield a crude product. Thin layer radiochromatography (TLRC) indicated a net activity of 89% associated with **4** and about 10% of the activity co-chromatographing with the starting N-methylbenzylamine. We found it convenient to purify **4** at this stage by high performance liquid chromatography (HPLC) and, after purification, the radiochemical yield for two runs was found to average 86%, based on [^{14}C]methylamine, with a radiochemical purity in excess of 98% as determined by TLRC.

The purified nitrile was hydrogenated at atmospheric pressure using Raney nickel as catalyst in ammoniacal ethanol to yield [methyl-^{14}C]-N-methyl-N-benzylputrescine (**5**) in a radiochemical yield of 70% based on **4**. TLRC of the product showed one radioactive peak co-chromatographing with reference **5**. Subsequent hydrogenation using 10% palladium on carbon as catalyst removed the benzyl protecting group and, upon acidification, gave [methyl-^{14}C]-N-methylputrescine dihydrochloride ([methyl-^{14}C]-**1**•2HCl) in radiochemical yield of 77% based on **5**. [Methyl-^{14}C]-**1** was stored as the dihydrochloride salt. TLRC of the amine **1** as well as the salt showed the product from one run to have a radiochemical purity in excess of 98%, and from a second run to be 97-98% radiochemically pure. In the latter case about 2% of the activity was associated with **4**. The radiochemical yield of [methyl-^{14}C]-**1** based on [^{14}C]methylamine was 26% and 38% for the two runs.

A one-step conversion of the nitrile **4** to [methyl-^{14}C]-**1** using palladium on charcoal at 50 psi was reported by Frydman, et al. (3) We noticed on occasion, however, a reaction by-product more polar than **1** and to avoid this, the two-step sequence was used.

EXPERIMENTAL

General. Physical and chemical properties of the intermediates and final product were determined on the non-radiolabelled materials. Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Varian XL-300 spectrometer. Melting points were determined on a Buchi 510 melting point apparatus. Preparative high performance liquid chromatography (HPLC) was done on a Whatman Magnum 9 Partisil 10/50 column using a Waters chromatographic system. Thin layer chromatography (TLC) was done using either silica gel 60 F-254 thin layer plates (Merck, 0.25 mm thickness) or cellulose thin layer plates (Merck, 0.1 mm thickness). Silica gel thin layer plates were developed in acetone/methanol/triethylamine (50/50/1.5) and cellulose thin layer plates

were developed in n-butanol/acetic acid/water (4/1/2). Chromatograms were visualized with ninhydrin spray reagent (Alltech Associates) followed by heating. Thin layer radiochromatography (TLRC) was carried out using either a Berthold TLC Linear Analyzer Model LB 2832 or a Radiomatic TLC Scanner Model RS.

[^{14}C]Methylamine hydrochloride (10 mCi, 59 mCi/mmol, 850 mCi/mg) was obtained from Amersham Corp. Benzaldehyde was distilled before use and stored in glass ampoules under nitrogen. Raney nickel (type 28, W. R. Grace) was washed successively with water and ethanol before use.

Preparation of [methyl-^{14}C]-N-methyl-benzylamine hydrochloride (**3**).

To a 25 mL 14/20 boiling flask was added [^{14}C]methylamine hydrochloride (0.46 mmol, 21.6 mCi/mmol) in 1 mL of water, a stirring bar and 1 mL of 0.5N potassium hydroxide. The flask was fitted with a greased short path distillation head and a 25 mL receiving flask containing benzaldehyde (47 mL, 0.46 mmol), 1 mL of ethanol and a stir bar. Positive pressure was maintained with argon and the receiving flask was cooled with a dry ice bath. The freed methylamine was then distilled as a solution. When the distillation was near completion, the flask was cooled to room temperature, 1 mL of water was added and the distillation continued. The receiving flask was fitted with a condenser and the reaction mixture was gently refluxed for 15 minutes. After cooling, the mixture was transferred to a 25 mL side arm flask containing 0.1 g of Raney nickel and a stirring bar. The flask was fitted with a pipette bulb and a rubber sleeve stopper, both of which were secured with copper wire. The reaction was stirred for two hours under an atmosphere of hydrogen. The catalyst was filtered off and the filtrate evaporated to half the volume. A 1 mL portion of 1N hydrochloric acid was added and the solution evaporated to dryness. The resulting solid was treated with 1 mL of ethanol and again concentrated to dryness *in vacuo* to give **3**. Thin layer radiochromatography (silica) indicated one major area of radioactivity (97%) with an Rf of 0.34.

Preparation of [methyl-^{14}C]-N-methyl-N-(3-cyanopropyl)benzylamine (**4**).

The crude [methyl-^{14}C]-N-methylbenzylamine hydrochloride (**3**) was transferred with 3 mL of water to a flask containing a stir bar, 200 mg of potassium carbonate and 45 mL of 4-bromobutyronitrile (0.45 mmol). The reaction mixture was stirred and heated under reflux for thirty minutes. After cooling to room temperature, the reaction mixture was extracted with ether (4x 5 mL), after which the ether extracts were combined, dried (sodium sulfate), filtered and concentrated. The resulting crude oil was then purified using HPLC (Partisil,

acetone/hexane/triethylamine, 10/90/0.8) to give 56.4 mg of **4** with a specific activity of 21.9 mCi/mmole (65% from [^{14}C]methylamine). TLRC (silica) indicated one radioactive area with an Rf of 0.75. 300 MHz ^{1}H NMR (CDCl): δ1.83 (2H, m), 2.18 (3H, s), 2.42 (2H, t, J=6.6 Hz.), 2.48 (2H, t, J=7.2 Hz.), 3.49 (2H,s), and 7.30 (5H,m); ir (neat): 2240 cm-1. Dipicrate, mp 98-99^{0}C.

Preparation of [methyl-^{14}C]-N-benzyl-N-methylputrescine (**5**).

The purified nitrile was reduced in an atmosphere of hydrogen using 0.1 g Raney nickel in 2 mL of dry ammonia saturated ethanol. After three hours, the sample was filtered and the solvent removed to yield **5** (70%). TLRC (cellulose) indicated one area of radioactivity with an Rf of 0.56 (Ninhydrin positive). 300 MHz ^{1}H NMR (CDCl): δ1.50 (6H, m), 2.18 (3H, s), 2.38 (2H, t, J=7.2 Hz.), 2.69 (2H, t, J=6.8 Hz.), 3.48 (2H,s), 7.30 (5H, m); Dipicrate, mp 148-150^{0}C, Anal. Calcd. for $C_{24}H_{26}N_8O_{14}$: C, 44.31; H, 4.03; N, 17.22 Found: C, 44.18; H, 4.07; N, 17.23.

Preparation of [methyl-^{14}C]-N-methylputrescine dihydrochloride ([methyl-^{14}C]-**1**•2HCl).

The [methyl-^{14}C]-N-benzyl-N-methylputrescine (**5**) was transferred with methanol to a 25 mL side arm flask containing 10% palladium on carbon and hydrogenated for 16 hours. The filtered sample was concentrated to half volume, 1 mL 1N hydrochloric acid added and then concentrated to dryness. The resulting solid was treated with 3 mL of ethanol and the solution again concentrated to dryness *in vacuo*. The [methyl-^{14}C]-N-methylputrescine dihydrochloride was dissolved in water and stored at 2^{0}C for later use. TLRC (cellulose) indicated one major area of radioactivity (99%) with an Rf of 0.30 (ninhydrin positive). The overall yield from the [^{14}C]methylamine was 26%. 300 MHz ^{1}H NMR (CDCl): δ1.52 (4H, m), 2.45 (3H, s), 2.60 (2H, t, J=6.7 Hz.), 2.73 (2H, t, J=6.9 Hz.). MS m/e : 102 (M+). Dipicrate, mp 229-230^{0}C (lit. (5) 229-231^{0}C); dihydrochloride, mp 181-182^{0}C (lit. (1) 179-181^{0}C).

ACKNOWLEDGMENTS

The authors would like to thank Dr. J. I. Seeman for helpful discussions and his interest in this work and J. D. Naworal for the mass spectral measurements.

REFERENCES

1. Leete, E., and McDonell, J. A. -J. Amer. Chem. Soc. 103: 658 (1981).
2. Mizusaki, S., Tanake, Y., Noguchi, M., and Tamaki, E. -Plant and Cell Physiol. 14: 103 (1973).
3. Alonso Garride, D. O., Buldain, G. and Frydman, B. -J. Org. Chem. 49: 2021 (1984) and references cited therein.
4. Feth, F., Arfmann, H. A., Wray, V., Wagner, K. C. -Phytochem. 24(5): 921 (1985).
5. Dudley, H. W., and Thorpe, W. V. -Biochem. J. 19: 845 (1925).