Synthesis of [^{14}C]-Pargyline (N-Methyl-N-([1-^{14}C]-propargyl)benzylamine) with the Radioactive Label on the Propargyl Group

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SUMMARY

A one-pot reaction scheme leading to ^{14}C-labeled pargyline (N-methyl-N- propargylbenzylamine) wherein the radioactive label from [^{14}C]-formaldehyde is synthetically incorporated into the methylene group of the propargyl side chain is described. Thus, [^{14}C]-formaldehyde (aqueous solution) is condensed in solvent dioxane with N-methylbenzylamine and mono-trimethylsilylacetylene in the presence of cupric acetate dihydrate at elevated temperatures (85-86°) for 24 hrs. The Mannich condensation product is then desilylated and the radiolabeled pargyline is purified by partitioning in ether from acid and base followed by flash chromatography, and isolated as the hydrochloride. The radioactive yield is 58%. This method should be generally applicable for the incorporation of radioactive label into the propargyl methylene group of other drugs containing a propargyl side chain.

KEY WORDS: [^{14}C]-Pargyline; inhibitors; monoamine oxidase; aldehyde dehydrogenase.

INTRODUCTION

Pargyline (1, N-methyl-N-propargylbenzylamine), whose hydrochloride salt (Eutonyl^{R}) is available clinically for the treatment of hypertension, is used experimentally as a monoamine oxidase inhibitor (1). Pargyline (1) is also a good inhibitor of aldehyde dehydrogenase (AlDH) in rodents, as manifested by elevation of ethanol-derived blood acetaldehyde (2,3). However, 1 does not inhibit AlDH *in vitro* and must be metabolically activated *in vivo* to generate the active enzyme inhibitor (4,5).

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Of the three possible metabolic reaction modes for the oxidative N-dealkylation of 1, viz., N-demethylation, N-debenzylation and N-depropargylation, only the last reaction produces propiolaldehyde (HC≡CCHO), a reactive α,β-acetylenic aldehyde, the putative active species that inhibits AlDH (5,6). Indeed, excessive metabolic generation of propiolaldehyde, e.g., as a result of induction of the hepatic microsomal cytochrome P-450 enzymes by agents such as phenobarbital, leads to hepatic necrosis (7), presumably due to glutathione depletion (8) and the resultant covalent binding of propiolaldehyde to tissue macromolecules.

^{14}C-Labeled pargylines, where the ^{14}C-label is on the methyl carbon (9) or on the methylene carbon of the benzyl group (10), have already been described. Methyl-labeled [^{11}C]-pargyline has also been prepared and used to study the biodistribution of pargyline in mice and rabbits by positron-emission tomography (11). In addition, ^{3}H-labeled pargyline (position of label not specified) has been used to study the labeling of the active site of monoamine oxidase (12). In order to quantitatively evaluate the tissue binding of pargyline-derived propiolaldehyde, it is necessary to selectively label the side-chain propargyl group with radioactive ^{3}H or ^{14}C. A Mannich reaction using 2-methyl-3-butyn-2-ol with formaldehyde and an appropriate secondary amine, followed by KOH catalyzed elimination of acetone from the resulting condensation product, has been used to synthesize not only pargyline but also clorgyline and L-deprenyl (13). This method was subsequently utilized for the synthesis of [^{14}C]-clorgyline and [^{14}C]-L-deprenyl using [^{14}C]-formaldehyde (14)†, but has not actually been applied to the synthesis of propargyl-labeled pargyline.

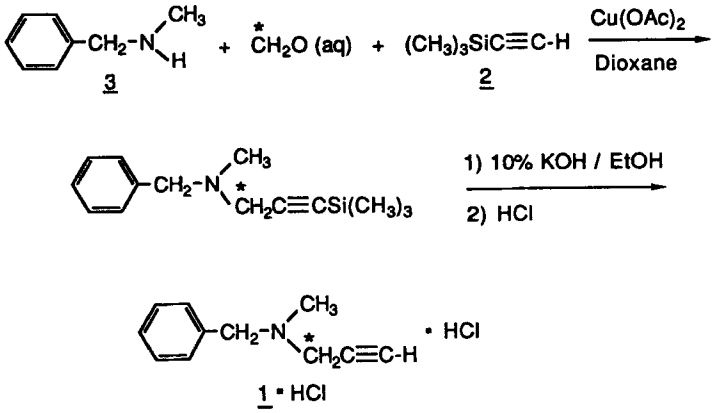
RESULTS AND DISCUSSION

We herewith present an alternative scheme using the readily deblocked trimethylsilylacetylene whereby the radioactive carbon label from [^{14}C-paraformaldehyde or [^{14}C]-formaldehyde can be incorporated directly into the propargyl side chain of 1. This one-pot synthesis is patterned after the known cupric acetate

† We are indebted to the reviewer for pointing out these references.

catalyzed condensation of mono-substituted acetylenes with paraformaldehyde and N,N-dialkyl or N-aryl, N-alkyl amines to give Mannich products (15), and is similar to the chlorgyline synthesis alluded to (14).

Scheme 1:



Accordingly, our synthetic procedure (Scheme 1) utilizes the partially protected mono-trimethylsilylacetylene (2) and methylbenzylamine (3), which are both commercially available. In cold runs, paraformaldehyde as the source of the one carbon fragment gave satisfactory yields of 1 (~40%). However, the unpredictability of the reaction with [^{14}C]-paraformaldehyde mandated the use of aqueous [^{14}C]-formaldehyde for the radioactive synthesis. The equivalence of this substitution was verified in cold runs. Hydrolysis of the trimethylsilyl protective group with ethanolic KOH (16), removal of acidic and neutral products by partitioning in ether, followed by separation of the product from the excess N-methylbenzylamine (3) by flash chromatography gave 1, which was isolated as its hydrochloride salt in 58% yield.

We believe that the present method for introducing a radioactive label (^{14}C or ^{3}H) on the propargyl side chain of pargyline may be universally applicable to other propargyl-bearing pharmacologic agents, such a pinazepam (17), 7-propargyltheophylline (18), 5-propargyloxyuridine (19), N^{10}-propargyl-5,8-dideazafolic acid and its analogs (20,21), and even to C-propargylglycine (22).

EXPERIMENTAL

[^{14}C]-Formaldehyde (aqueous) and Aquasol^{R} were obtained from Dupont NEN Research Products. Mono-trimethylsilylacetylene (2) was purchased from K & K Laboratories of ICN Biochemicals and was redistilled before use. N-Methylbenzylamine (3) and $Cu(OAc)_2 \cdot H_2O$ were purchased from Aldrich Chemical Co. The adsorbent for flash chromatography (Kieselgel 60) was a product of EM Science. All solvent evaporations were conducted using a rotary evaporator attached to a water aspirator. Specific radioactivity of the final products were determined in Aquasol^{R} in a Packard 4640 liquid scintillation spectrometer and corrected for quenching.

N-Methyl-N-(1-[^{14}C]-propargyl)benzylamine (1).

Into a glass, pressure reaction vial (1.0 i.d. x 8.0 cm) with a teflon-lined screw cap was placed 37% aqueous formaldehyde (82 mg, 30 mg CH_2O, 1.0 mmol), dioxane (0.25 mL), N-methylbenzylamine (3, 0.15 g, 1.2 mmol), mono-trimethylsilylacetylene (2, 0.12 g, 1.2 mmol), [^{14}C]-formaldehyde (1.0 mCi, 55.0 mCi/mmol), dioxane (2.25 mL) and $Cu(OAc)_2 \cdot H_2O$ (25 mg) in that order. The second volume of dioxane was used to rinse the tube that contained the radiolabeled formaldehyde. After inserting a magnetic stirring bar, the tube was capped and heated at 84-86°C for 24 h with stirring. The reaction turned green within the first ten minutes of heating and was brown (with brown solids) after heating was terminated. The mixture was filtered (suction) through Celite, the filter cake washed with ether, and the filtrate and washings were concentrated. A solution of KOH (0.54 g, 9.6 mmol) in 95% ethanol (5 mL) was added to the residue, and the resulting solution was stirred at room temperature for 5.5 h to hydrolyze the trimethylsilyl group. After concentration to about 2 mL, H_2O (5 mL) was added to the residue and the mixture was acidified (pH <2) with 2 M HCl. This solution was extracted with ether (2 x 10 mL) to remove acidic and neutral by-products and then made basic (pH >11) with 6 M NaOH. The resulting mixture was extracted with ether (3 x 10 mL), and the combined ether extracts were dried over Na_2SO_4 and concentrated. The residual brown liquid was then applied to a column of Kieselgel 60 (2.8 x 12 cm, 230-400 mesh) packed in ethyl acetate for

flash chromatography, and the column was eluted with ethyl acetate at 10 psi, collecting fractions of about 10 mL. The individual fractions were monitored for radioactivity and by TLC, the results of which indicated that the desired product was located mainly in fractions 5-7. Fractions 4-7 were therefore combined and concentrated; absolute ethanolic HCl was then added to the residue and the solvent was again removed. The resulting tan solids were dissolved in absolute EtOH, and the solution was decolorized with activated carbon. Crystallization from absolute EtOH-ether yielded 114 mg (58%) of 1·HCl as white solids, specific radioactivity = 0.928 mCi/mmol. Another crop of radiolabeled product of lower specific radioactivity was obtained by adding non-radioactive pargyline hydrochloride (51 mg) to the filtrate from the first crop, concentrating the solution to a small volume, and crystallization from absolute EtOH-ether (44 mg, specific radioactivity = 0.173 mCi/mmol). Total recovery of radioactivity = 0.58 mCi (58%).

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