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RAPID SYNTHESIS OF RADIOLABELED PHENYLETHANOLAMINE

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SUMMARY

A method for the rapid synthesis of [^{14}C]-phenylethanolamine (PEOH) amenable to the preparation of [^{11}C]-PEOH was developed under anhydrous conditions. Reaction of benzaldehyde with [^{14}C]-NaCN in the presence of crown ethers in THF yielded [^{14}C]-cyanohydrin which was reduced to [^{14}C]-PEOH with borane in the same solvent. The overall chemical and radiochemical yields were 33%.

KEY WORDS: Radiolabeled phenylethanolamine, cyanohydrin, benzaldehyde, crown ethers, radiochemical yield.

INTRODUCTION

Biogenic amines have been shown to play an important role in the regulation of brain function (1). A renewed interest in phenylethanolamine (PEOH) has developed because of its similarity in structure and pharmacological profile to amphetamine, and because of the detection of trace amounts in mammalian peripheral tissues and brain (2-4). Labeling compounds with carbon-11 allows a convenient method for studying their *in vivo* disposition by non-invasive methodology.

The synthesis of [^{11}C]-labeled amines is commonly achieved by the displacement reaction on alkyl halides by [^{11}C]-labeled sodium cyanide followed by reduction of the resulting labeled nitrile (5). For example, carbon-11 labeled norepinephrine (a compound structurally similar to PEOH) was synthesized (6) by reaction of [^{11}C]-NaCN with the sodium bisulfite addition product of 3,4-dihydroxybenzaldehyde in water with heating. The solution was extracted with ether and the extracted 2,3-dihydroxycyanohydrin was then reduced with borane to the desired amine.

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In our laboratory an alternative method was developed and used in the synthesis of [α -^{14}C]-phenethylamine (7). The method utilized crown ethers for the solubilization of cyanide in tetrahydrofuran (THF) for the formation of phenylacetonitrile from benzyl chloride. Reduction of phenylacetonitrile to phenethylamine was accomplished with borane using the same solvent.

In this report a method is described which uses crown ethers for the solubilization of [^{14}C]-NaCN in THF permitting the conversion of benzaldehyde to labeled cyanohydrin. The reduction of the cyanohydrin to [^{14}C]-phenylethanolamine is accomplished in the same solvent and reaction vessel. The method is rapid and amenable to the synthesis of [^{11}C]-PEOH.

EXPERIMENTAL

Reagents

2-Amino-1-phenylethanol (phenylethanolamine), sodium cyanide, benzaldehyde, 18-crown-6, 15-crown-5, tetrahydrofuran (gold label) and borane: tetrahydrofuran complex were purchased from Aldrich Chemical Company. Ninhydrin was purchased from Sigma Chemical Company. Sodium hydroxide, sodium chloride, ammonium hydroxide and ammonium acetate (HPLC grade) were purchased from Fisher Scientific Company. Cation-exchange resin (AG50W-X2) was purchased from Bio-Rad Laboratories. [^{14}C]-Sodium cyanide (7.9 mCi/mmol) and Aquasol-2 were purchased from New England Nuclear Corp. LK5F silica gel thin layer chromatography plates were purchased from Whatman, Inc. Cab-O-Sil (grade 5) was purchased from Cabot Corp. Methanol (HPLC grade) was purchased from MCB Manufacturing Chemists, Inc.

Synthetic Procedure for [^{14}C]-Phenylethanolamine

A 1.0 ml aliquot of NaCN in 0.005 N NaOH (2 mg, 4 x 10^{-2} mmol) and [^{14}C]-NaCN in 0.005 N NaOH (3.6 μCi , 7.9 mCi/mmol) were placed in a beaker and the water was completely evaporated on a hot plate. A mixture of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6) (1.25 g, 4.7 mmol), 1,4,7,10,13-pentaoxacyclopentadecane (15-crown-5) (0.25 g, 1.1 mmol) and tetrahydrofuran (THF) (3 ml) were added to the beaker which was covered with parafilm and placed in an ultrasonic bath for 10 min. This combination of 18-crown-6 and 15-crown-5 provides the greatest solubility of cyanide in THF (7). Benzaldehyde (93.7 mg, 0.88 mmol) was added to the above solution and the entire mixture was sonicated for an extra 5 minutes and transferred to a three-neck flask (preheated to 80°C using a heating mantle). The flask was fitted with an addition funnel containing 3 ml of 1M borane in tetrahydrofuran (BH_3/THF). The borane solution was added dropwise with stirring to the reaction mixture over a period of 2 min. Heating with reflux was continued for about 10 min. after which the system was connected to an NaOH trap (100 ml, 0.2 N). Excess borane was destroyed by the slow addition from the addition funnel of a portion of 1N HCl (18 ml) containing NaCl (0.33 g, 5.8 mmol). After the vigorous evolution of H_2 gas ceased, the remainder of the HCl solution was added rapidly. Nitrogen gas was then bubbled through the quenched reaction mixture for 20 min. This trapping of [^{14}C]HCN is not needed for carbon-11 synthesis. The contents of the three-neck flask were passed through a cation-exchange resin packed in a 1.9cm x 51cm column and was prewashed with 1N HCl (100 ml). The column was then washed with H_2O (30 ml)

and 0.2 N NaOH until the resin was neutral as indicated by a darkening of the resin. The next 40 ml of the basic eluate were collected; in this volume the amine was contained. This basic eluate was then acidified, lyophilized and reconstituted in distilled water (2 ml).

Thin Layer Chromatography

The water-reconstituted product was qualitatively analyzed by thin layer chromatography on a Whatman LK5F silica gel plate (25 x 5 cm). The reconstituted product was spotted along the base of the TLC plate and developed with ethanol:ammonium hydroxide (4:1). After the plate was developed and dried, one side of the plate was covered with a sheet of paper and the exposed portion was sprayed with ninhydrin solution (1,2,3-indantrione monohydrate) in ethanol (1 mg/ml). The ninhydrin reactive spot with an Rf value of 0.72 corresponded to the R_f value exhibited by authentic phenylethanolamine when chromatographed under identical conditions. A radiochromatogram was generated by scraping 1.5 cm strips from the side of the plate not sprayed with ninhydrin, suspending the silica gel in Cab-O-Sil and Aquasol-2 cocktail and counting in the liquid scintillation counter.

HPLC Analysis

High performance liquid chromatography was carried out using a solvent delivery pump,[1] and a loop injector[2] with a 20 µl sample loop, a variable wavelength detector[3] and strip chart recorder.[4] Separation was achieved at ambient temperature on a 25 cm x 4.6 mm stainless steel column prepacked with 5µ octadecylsilane particles.[5] Mobile phase consisted of 33% (v/v) methanol in 0.1M ammonium acetate delivered at 1 ml/min. Absorbance was read at 254 nm. At a flow rate of 1 ml/min., phenylethanolamine eluted at 5.5 min.

A standard curve of authentic phenylethanolamine was constructed by plotting concentration (mg/ml) versus peak height (cm). The lyophilized water-reconstituted product was injected into the HPLC system. One ml fractions were collected in scintillation vials and counted in aquasol-2 liquid scintillation cocktail in the liquid scintillation counter. A plot of radioactivity (dpm) versus retention time was constructed. The concentration of [^{14}C]-phenylethanolamine was calculated by fitting the peak height into the standard curve. Using the radioactivity corresponding to the phenylethanolamine peak, the radiochemical yield was calculated.

RESULTS AND DISCUSSION

To avoid time-consuming isolation and extraction steps, a "one-pot" synthesis of phenylethanolamine was accomplished by the reaction of benzaldehyde and [^{14}C]-NaCN in THF (using crown ethers to solubilize the Na^{14}CN), and reduction of the resulting cyanohydrin with BH_3/THF as shown below.

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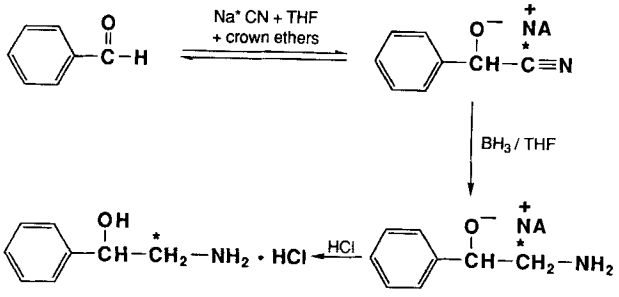
[1] Pump: Altex, model 110A, Beckman Instruments, Inc.

[2] Injector: Rheodyne model 7125.

[3] Detector: Varichrom; Varian.

[4] Recorder: Omniscribe recorder; Houston Instruments.

[5] Column: Ultrasphere-ODS-5 µm; Altex, Beckman Instruments.



The identity of the product was determined by TLC, which showed that all the radioactivity appeared at the R_f value corresponding to authentic PEOH. The chemical and radiochemical yields were determined by HPLC using u.v. detection and liquid scintillation counting. The chemical yield (based on NaCN) was found to be 33% and the radiochemical yield (based on [^{14}C]-NaCN) was also 33%. Using the herein reported procedure one could accomplish, with relatively high yields, the synthesis of [^{11}C]-labeled PEOH within 60 minutes, which is compatible with the half-life (20 min.) of carbon-11.

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