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Syntheses of *N-t*-Butyl-α-phenylnitrone-α-^{14}C and α-(4-Pyridyl-1-oxide)-*N-t*-butylnitrone-α-^{14}C.

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SUMMARY

N-t-Butyl-α-phenylnitrone-α-^{14}C was prepared from benzoic-α-^{14}C acid via benzyl alcohol in 40 - 55% yield (sp. activity: 30 mCi/mmol). α-(4-Pyridyl-1-oxide)-*N-t*-butylnitrone-α-^{14}C arrived from $Ba^{14}CO_3$ via carboxylation of 4-bromopyridine in 7 - 22% yield (sp. activity: 23 mCi/mmol). Both radiolabelled spin traps showed >99% radiochemical purity by HPLC and suffered <1% loss after 1 - 1.5 months storage under the conditions specified.

Keywords: *N-t*-Butyl-α-phenylnitrone-α-^{14}C; α-(4-pyridyl-1-oxide)-*N-t*-butylnitrone-α-^{14}C.

INTRODUCTION

Widely used nitrone spin traps α-phenyl-*N-t*-butylnitrone (PBN) and α-(4-pyridyl-1-oxide)-*N-t*-butylnitrone (4-POBN) exhibit some promising therapeutic and prophylactic activities, including lowering septic shock fatality, reversing the effects of aging, cardioprotection and vasodilatation in ischemia/reperfusion models of myocardial infarction[1]. Further pharmacological evaluation of these potentially useful nitrones may benefit from a ready availability of their ^{14}C-labelled analogs.

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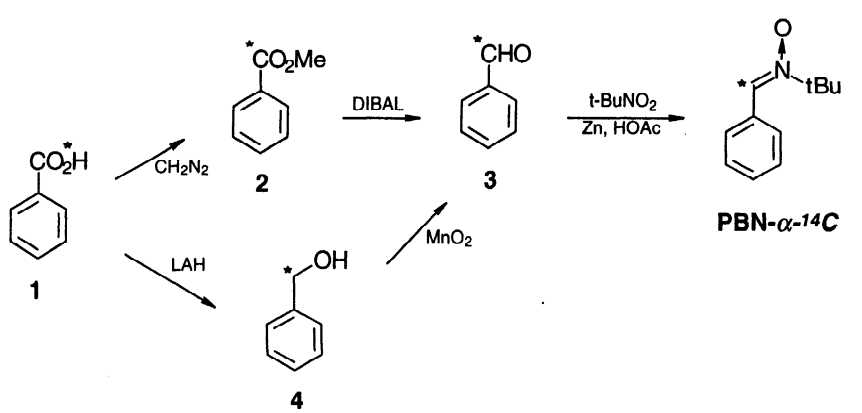
RESULTS AND DISCUSSION

Developmental cold runs suggested the labelled PBN can arrive via two slightly different routes. Esterification of benzoic **1** with diazomethane (Scheme I) followed by reduction of ester **2** with DIBAL produced the desired benzaldehyde **3**. The methylation was facile, clean, and in high yield as expected, but the reduction gave only 45 - 55% yield and also some by-products. In contrast, reduction of **1** with $LiAlH_4$ and subsequent oxidation of alcohol **4** with MnO_2 proceeded cleanly to give the desired aldehyde **3** in high purity and yields (84% based on **1**). Fujisawa *et al.*[3] reported a one-pot conversion of benzoic acid into its aldehyde (78% yield by GC) using chloromethylidene-*N,N*-dimethyliminium chloride and $LiAlH(OtBu)_3$. Nonetheless, the $LiAlH_4$-MnO_2 route shown herein remains preferable due to its higher yield, pure crudes, and simple procedures.

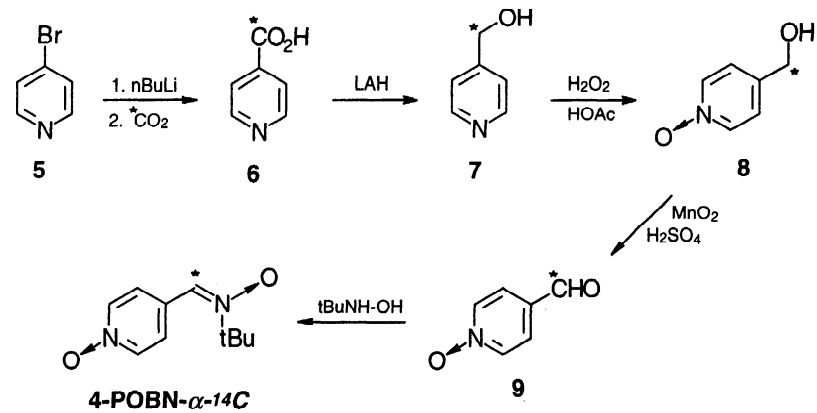
Crude benzaldehyde so obtained was suitable for the next condensation with *t*-nitrobutane, adapting the method by Huie and Cherry[2]. Thus, PBN-α-^{14}C was obtained in 40 - 55% overall yield from **1** in 99% purity by HPLC (sp. activity: 30 mCi/mmole). When stored at -20°C in ethanol at a concentration of 0.1 mCi/mL (0.591 mg/mL), the radioactive nitrone showed via HPLC analysis a 1% loss in purity after 1 month. Synthesis of *N-t*-Butyl-α-phenylnitrone-*[U-ring]*-^{14}C via coupling of labelled benzaldehyde and *N-t*-butylhydroxylamine has also been reported[4].

Scheme I.



Nitrone 4-POBN-α-^{14}C was prepared as shown in Scheme II. Lithiation of bromopyridine **5** with *n*BuLi followed by carboxylation with $^{14}CO_2$, generated from $Ba^{14}CO_3$ and concentrated H_2SO_4, afforded labeled compound **6** in 70 - 80% yield. Murray and Langham[5] and others[6] have similarly synthesized the labeled isonicotinic acid. Reduction of **6** with $LiAlH_4$ gave carbinol **7** in yields ranging from 20 - 56%. It appears the carbinol is highly water-soluble; and more effective extraction techniques during aqueous work-up led to better yields. Conversion of **7** into the target 4-POBN-α-^{14}C followed the same sequence of *N*-oxygenation, oxidation, and then condensation with *t*BuNH-OH as reported for the unlabeled compound[7] (15 - 55% yield from **7**). The labelled nitrone so produced has a 99.9% radiochemical purity by HPLC (sp. activity: 23 mCi/mmol). When stored at -20°C in ethanol at a concentration of 0.1 mCi/mL (0.896 mg/mL), the compound showed via HPLC analysis a slight 0.6% loss in purity after 1.5 months.

Scheme II.



EXPERIMENTAL SECTION

General. Reactions involving moisture-sensitive reagents were carried out under anhydrous conditions, which signify drying glassware in ovens at 130°C and

cooling under N_2, freshly distilling solvents over a desiccating agent under N_2 or using purchased anhydrous-grade materials, and blanketing the reaction mixture with N_2. The structures of all products during developmental cold runs were confirmed via the usual spectroscopic data and physical properties. Melting points were taken with a Mel-Temp II and uncorrected. IR spectra were recorded on a Beckman Acculab 4 or Mattson Galaxy 3000 FT-IR. ^1H and ^{13}C NMR were obtained with either a Bruker AC-250 NMR or Varian Unity 300 NMR. GC-MS were recorded with a Hewlett Packard 5995 using the electron impact method unless specified otherwise.

Benzyl-α-^{14}C Alcohol. To a suspension of 0.084 g $LiAlH_4$ (2.2 mmol) in 4 mL anhydrous Et_2O at 5°C was added an ethereal solution of benzoic-α-^{14}C acid (60.0 mCi; 30 mCi/mmole; 10 mL). The resulting mixture was then stirred at 20°C for 15 hr, cooled back to 5°C, quenched slowly with 0.1 mL water, 0.1 mL of 15% NaOH, and then 0.25 mL water. Stirring continued for another 30 min followed by filtration to remove the white precipitate. The ether filtrate was washed once with 5 mL of saturated $NaHCO_3$ and twice with 5 mL of brine, dried over $MgSO_4$, and evaporated to give 58 mCi of product (97% yield).

Benzaldehyde-α-^{14}C. To the crude benzyl alcohol (58 mCi; 30 mCi/mmole) in 15 mL of refluxing dry pentane was added all at once 0.85 g activated powder MnO_2 (9.74 mmol). Stirring continued for 5 hr at 40°C, followed by filtration and washing with pentane (4 x 5 mL). Evaporation of the combined pentane at 20°C in a rotary evaporator gave 48.6 mCi of crude benzaldehyde (84% yield).

***N-t*-Butyl-α-phenylnitrone-α-^{14}C.** To a mixture of the crude benzaldehyde (48.6 mCi; 30 mCi/mmole; 1.62 mmol) and 0.320 g 2-methyl-2-nitropropane (3.1 mmol) and 0.348 g zinc dust (5.31 mmol) in 12.5 mL 95% ethanol at 0 - 5°C was added 0.63 mL glacial acetic acid (10.4 mmol) slowly over 50 min under vigorous stirring. The resulting mixture was stirred for 5 hr at 5°C, let stand in the refrigerator for 3 days,

filtered to remove the zinc salts, stripped of solvents, and then dried under high vacuum for 1 hr. The residue was taken up in 25 mL ether, washed with 10 mL water, dried over $MgSO_4$, and evaporated to dryness. The crude was then redissolved in 10 mL EtOH and millipored to afford 24 mCi of pure product. TLC (SiO_2; 80:1 CH_2Cl_2/MeOH): one spot at $R_f = 0.4$. Reverse phase HPLC (Supelco Discovery C-18 column, 4.6 x 250 mm, 15% EtOH in water mobile phase, 1.0 mL/min flow rate): 99% purity.

Isonicotinic-α-^{14}C Acid. A solution of 1.61g of 4-bromopyridine hydrochloride (5.4 mmol) in 4 mL of water was made basic with saturated Na_2CO_3 and extracted with Et_2O (3 x 10 mL). The ethereal solution was dried over $CaSO_4$ for 1 day and 3A° sieves for another day, filtered, cooled to -78°C, stirred with 1.7 mL of 2.5M *n*BuLi (4.2 mmol) for 30 min, further cooled to -178°C (liquid nitrogen), exposed to $^{14}CO_2$ generated from $Ba^{14}CO_3$ (85 mCi; 40 mCi/mmole) and conc. H_2SO_4 while warming up to -78°C, stirred for 1.5 hr, and then quenched with 25 mL water. The aqueous layer was washed with ether (3 x 15 mL). The combined ether layers were back extracted once with water. The combined aqueous solution was acidified with 6N HCl and washed again with ether (3 x 5 mL) and then evaporated to dryness. The residue was dissolved in 6.5 mL of water and adjusted to pH 3 with $NaHCO_3$ to produce 0.185 g of crystalline product (60 mCi; 70% yield).

4-Pyridylcarbinol-α-^{14}C. To the isonicotinic acid obtained above (60 mCi; 40 mCi/mmol) in 6.5 mL THF at 5°C was added 0.119g $LiAlH_4$ (3.1 mmole). The resulting mixture was stirred for 15 hr at 20°C, cooled back to 5°C, quenched in sequence with 0.14 mL of water, 0.14 mL 15% NaOH, and then 0.42 mL water. The white precipitate was filtered off and washed with CH_2Cl_2 (5 x 15 mL). The combined organic solution was concentrated to dryness. The residue was taken up in 40 mL of CH_2Cl_2, washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and stripped of solvents to give 12 mCi of crude product (20% yield).

4-Pyridylcarbinol-*N*-oxide-α-^{14}C. To the above pyridylcarbinol (12 mCi; 40 mCi/mmole) dissolved in 1.0 mL acetic acid was added 0.3 mL of 30% H_2O_2. The resulting solution was stirred at 80°C for 5 hr and then treated with another 0.3 mL of 30% H_2O_2 for 10 hr at 80°C, concentrated under vacuum, made alkaline with 0.3 g K_2CO_3, diluted with 15 mL $CHCl_3$, stirred and heated for 10 min, filtered to remove insoluble solid, and then evaporated to give the crude oxide (7.5 mCi; 62% yield).

4-Pyridinecarboxaldehyde-*N*-oxide-α-^{14}C. To the crude *N*-oxide obtained above (7.5 mCi; 23 mCi/mmole) dissolved in 9 mL $CHCl_3$ was added all at once 0.203 g activated powder MnO_2 (2.3 mmol). The resulting mixture was refluxed with vigorous stirring for 10 hr, cooled to 20°C, filtered (solid washed with $CHCl_3$), and concentrated under vacuum. Flash LC of the residue (silica gel, 10:1:0.3 EtOAc/MeOH/HOAc) gave 6.7 mCi of the aldehyde (89% yield).

α-(4-Pyridyl-1-oxide)-*N*-*t*-butylnitrone-α-^{14}C. A mixture consisting of the aldehyde-*N*-oxide obtained above (6.7 mCi; 23 mCi/mmol), 0.039 g *N*-butylhydroxylamine (0.3 mmol), 4A° molecular sieves, 10 mL $CHCl_3$, and 0.03 mL 50% NaOH solution was stirred at 20°C for 24 hr and then filtered. The filtrate was concentrated and then chromatographed (silica gel, 7:2:1 EtOAc/ MeOH/HOAc) to produce 2.9 mCi of the nitrone at about 80% purity. Further purification by C-18 reversed phase HPLC (10% EtOH in water) afforded 1.8m Ci of pure product.

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