SYNTHESIS OF 3-AMINO-1-METHYL-5H-PYRIDO[4,3-b]INDOLE-1-^{14}C (TRP-P-2)

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SUMMARY

A practical 2-step synthesis of 3-amino-1-methyl-5H-pyrido[4,3-b]indole-1-^{14}C is described, starting from 2-cyanomethyl indole and carbonyl-labeled acetyl chloride.

Key Words: TRP-P-2, 3-hydroxy-1-methyl-5H-pyrido[4,3-b]indole, 3-amino-1-methyl-5H-pyrido[4,3-b]indole-1-^{14}C.

INTRODUCTION

The pyrolysis of amino acids or protein foods is known to contribute a significant source of mutagens to the diet.[1] Pyrolysis products from tryptophan exhibit mutagenic activities in the Ames assay that are as high as or higher than previously known promutagens such as aflatoxin B_1 and benzo[a]pyrene. One of the most potent from this class is 3-amino-1-methyl-5H-pyrido[4,3-b]indole (TRP-P-2) (4). In order to study the metabolism and disposition of TRP-P-2 in the whole animal, we have prepared TRP-P-2 labeled at C-1, a position that is unlikely to be lost during its intermediary metabolism. Its synthesis is described in this publication.

DISCUSSION

A synthetic route that lends itself to the synthesis of TRP-P-2-1-^{14}C starts from the known 2-cyanomethyl indole (1).[2] Introduction of a 2 carbon fragment at C-3 followed by cyclization can give the desired product. Two syntheses of TRP-P-2 starting from 1 have been described. In one,[3] 2-cyanomethylindole is converted to 3-acetyl-2-cyanomethylindole (3) using dimethyl acetamide and phosphoryl chloride by means of a modified Vilsmeier-Haack reaction.[4] Treatment of 3 with methanolic ammonia was reported to give the desired TPP-P-2, however no yields were given. This procedure has been used to prepare ^{14}C labeled TRP-P-2,[5] although again no yields were reported. A second synthesis starting from 2-cyanoindole gives TRP-P-2

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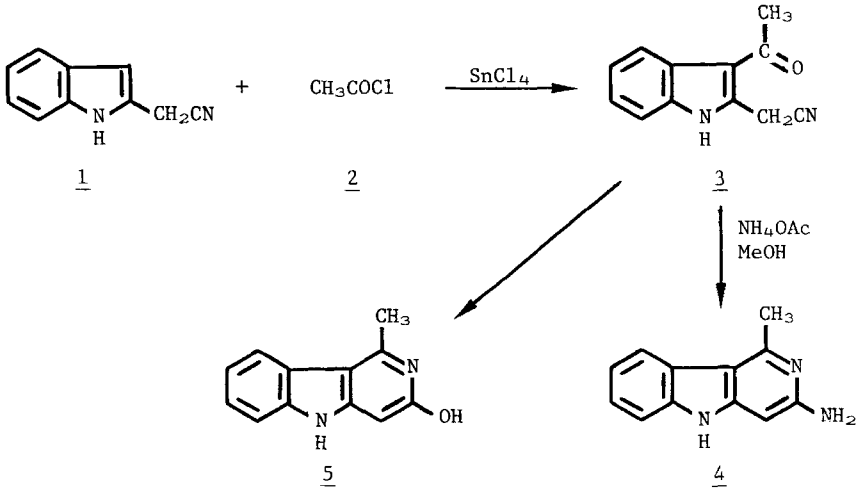
in 1 step by a Friedel-Crafts reaction using acetonitrile as reactant and solvent.[6] The yield reported was 10% based on 1, however, the use of acetonitrile as both reactant and solvent made this approach impractical for the preparation of a ^{14}C-labeled derivative.

We found that a modified Friedel-Crafts reaction of 2-cyanoindole with 1.7 equivalents of acetyl chloride in benzene using stannic chloride catalysis (7) gave a 45% yield of 3-acetyl-2-cyanomethylindole (3). This yield represents a 25% incorporation of acetyl chloride. In our experience, this procedure results in the most practical synthesis of acetyl labeled 3-acetyl-2-cyanomethyl indole (3), the key radiolabeled intermediate for the synthesis of TRP-P-2-1-^{14}C. This reaction proved to be reproducible at scales ranging from 5 g for the preparation of quantities of unlabeled 4 to 500 mg for the radiolabeled run.



The reaction conditions for the cyclization of 3 using methanolic ammonia proved to be critical. Treatment of 3 with methanolic ammonia at room temperature gave no reaction, while at 70-80° extensive decomposition occurred and no identifiable product could be isolated. The use of ammonium acetate in methanol, however, was satisfactory for the ring closure, although, again, the temperature of reaction was critical. At 65° a 1:1 mixture of TRP-P-2 (4) and a by-product that proved to be 3-hydroxy-1-methyl-5H-pyrido[4,3-b]indole (5) was obtained. At 78° (refluxing

ethanol) only the by-product (5) was isolated. At 45° a 31% yield of TRP-P-2 (4) was obtained as the acetate salt after chromatogrpahy and recrystallization.

The cyclization reaction at 45° also proved to be reproducible on a scale ranging from 2.6 g of unlabeled 3 to 347 mg of labeled 3.

EXPERIMENTAL

Acetyl chloride-1-^{14}C, purchased from Wizard Laboratories, Davis, California, was redistilled immediately before use and had a specific activity of 3 mCi/mM. Thin-layer chromatograms were run on Analtech Silica Gel GF using ethyl acetate/ethanol/acetic acid (47:47:6) for the developing solvent (System 1), or 20% ethyl acetate/80% chloroform for the developing solvent (System 2).

3-Acetyl-2-cyanomethylindole (3)

To a suspension of 4.50 g (28.8 mM) of 2-cyanomethylindole (1)[2] and 3.6 ml (50.4 mM) of acetyl chloride (2) in 70 ml of dry benzene at 5° was added a solution of 5.85 ml of stannic chloride in 20 ml of dry benzene, dropwise with stirring and continued cooling. The solution was stirred for 1 hr at 5°, then was poured into 200 ml of ice water. The 2-phase mixture was stirred for 30 min and then was filtered. The solid product was dried under vacuum, then recrystallized from 130 ml of acetone to give 3.1 g (54%) of 3-acetyl-2-cyanomethylindole (3), m.p. 215-218°. The thin layer chromatogram in system 2 showed 1 spot at R_f 0.38.

NMR (DMSOd_6) δ2.64 (3 H, s, CH_3), 4.48 (2 H, s, CH_2CN) 7.1-8.1 (4 H, m, aromatic). Akimoto et al.[3] reported m.p. 223-225°.

The radiolabeled run was carried out using 682 mg (4.36 mmoles) of 2-cyanomethylindole, 582 mg (7.42 mmoles) of redistilled acetyl chloride-1-^{14}C (3 mCi/mmole), 0.868 ml (7.42 mmoles) of stannic chloride and 12 ml of dry benzene to give 347 mg (40%) of labeled 3 that was homogeneous in system 2 and identical in R_f with unlabeled material.

3-Amino-1-methyl-5H-pyrido[4,3-b]indole (TRP-P-2) and 3-hydroxy-1-methyl-5H-pyrido-[4,3-b]indole (5)

A mixture of 2.6 g of 3-acetyl-2-cyanomethylindole and 20 g of ammonium acetate in 400 ml of methanol was stirred and heated at 45° for 10 days. The methanol was

evaporated in vacuo. The residue was dissolved in 1 liter of water and filtered. The filtrate was treated with concentrated aqueous ammonia to pH 10 and extracted with 2 x 500 ml of dichloromethane and 2 x 500 ml of ethyl acetate. The combined organic layers were dryed ($MgSO_4$) and evaporated to a brown solid. The solid was dissolved in 25 ml of ethyl acetate containing 2.5 ml of methanol then 1 ml of acetic acid was added. The resulting precipitate was filtered and dried to give 1.02 g (31%) of material that was homogeneous on TLC in system 1 with R_f 0.31.

Recrystallization from methanol-ethyl acetate (1:4) gave 0.92 g (28%) of product with m.p. 230-235°.

NMR (DMSOd_6) δ 1.90 (3 H, s, acetate), 2.72 (3 H, s, CH_3), 6.28 (1 H, s, H-4), 7.00-7.40 (3 H, m, aromatic), 7.84 (1 H, m, aromatic), 11.11 ppm (1 H, br s, NH); mass spectrum m/e 197 (M^+).

The radiolabeled run was carried out using 347 mg of ^{14}C labeled 3-acetylindole-2-acetonitrile (3) and 3.47 g of ammonium acetate in 50 ml of methanol. The product (4), obtained after chromatography and two recrystallizations from methanol-ethyl acetate (1:4) weighed 127 mg (28%) and had specific activity of 3.0 mCi/mmole.

A radioautogram of 4 using System 1 showed one spot at R_f 0.31.

When the reaction temperature was raised to 76°, 3-hydroxy-1-methyl-5H-pyrido-[4,3-b]indole predominated. Thus a solution of 2-cyanomethyl-3-acetylindole (50 mg, 0.25 mmoles) and ammonium acetate (500 mg) in 10 ml of absolute ethanol was refluxed for 24 hours under argon. The solvent was evaporated and the residue chromatographed on silica gel with 50% EtOH/50% EtOAc to give 23 mg (46%) of 3-hydroxy-1-methyl-5H-pyrido[4,3-b]indole. The thin layer chromatogram in system 1 showed 1 spot at R_f 0.58. There was no trace of TRP-P-2 at R_f 0.31.

NMR (DMSO-d_6) δ 2.65 (3 H, s, $-CH_3$), 5.73 (1 H, s, H-4), 7.02-7.23 (3 H, m, arom), 7.72 (1 H, br d, H-5); mass spectrum, m/e 198 (M^+).

These data are compatible with the nmr and mass spectra that were reported by Pezzuto et al.[8]

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