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PREPARATION OF CARBON-14 LABELED N-(2-ETHYL-3-CHLORO-4-PYRIDINYL)-4-(4-CHLOROPHENOXY)PHENYLACETAMIDE

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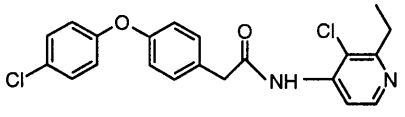
SUMMARY

A 4-step modified route has been developed for the synthesis of N-(2-ethyl-3-chloro-4-pyridinyl-2-[^{14}C])-4-(4-chlorophenoxy)phenylacetamide ([^{14}C]-**1**). The key transformation in the sequence is an amination/cyclization pair of reactions that furnished 2-[^{14}C]-4-amino-2-ethylpyridine (**4**) from 5-[^{14}C]-1-methoxyhept-1-en-3-yn-5-one (**3**) and ammonia. Amine **4** was then selectively chlorinated to provide 2-[^{14}C]-4-amino-3-chloro-2-ethylpyridine (**5**) which was then coupled with 4-(4-chlorophenoxy)phenylacetic ethylformic anhydride (**6**) in a reaction mediated by trimethylaluminum to furnish the desired, radiolabeled phenylacetamide [^{14}C]-**1**.

Key Words: N-(2-ethyl-3-chloro-4-pyridinyl-2-[^{14}C])-4-(4-chlorophenoxy)phenylacetamide, 2-[^{14}C]-4-amino-2-ethylpyridine, 2-[^{14}C]-4-amino-3-chloro-2-ethylpyridine, 5-[^{14}C]-1-methoxyhept-1-en-3-yn-5-one

INTRODUCTION

N-(2-Ethyl-3-chloro-4-pyridinyl)-4-(4-chlorophenoxy)phenylacetamide (**1**) is a compound with interesting insecticidal activity (1). In order to fully characterize its biological behavior a radiolabeled sample was requested for biochemical mode of action, metabolism and transport studies. Because of the ease of synthesis, cost of radiochemical starting materials and perceived metabolic stability, it was decided to place the carbon-14 label at the 2-position of the pyridine ring.



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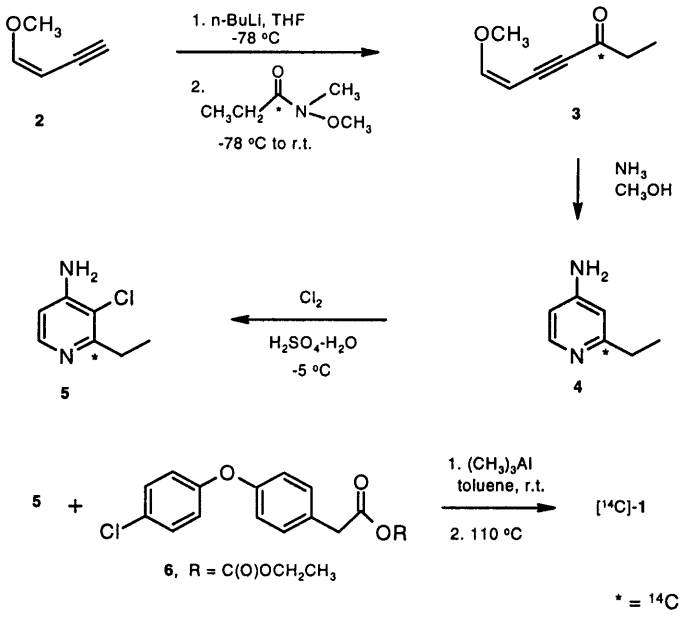
RESULTS AND DISCUSSION

The preparation of [^{14}C]-**1** is described below in the Scheme. The synthetic route chosen to prepare aminopyridine **4** was a modification of a method developed previously (2). Methoxyenyne **2** was lithiated with n-butyllithium in THF and the resulting lithium acetylide was treated with 1-[^{14}C]-N-

Scheme



methoxy-N-methylpropanamide using the conditions described by Weinreb (3) to furnish, after acid workup, acetylenic ketone **3** in virtually quantitative yield. Efficient formation of **3** was obtained by using two equivalents of the lithium acetylide (to consume any adventitious water) and by using the Weinreb-type amide rather than propionic anhydride, as had been used previously (2). Use of the Weinreb amide approach mitigated the possibility of obtaining any additional products derived from the reaction of ketone 3 with the excess lithium acetylide.

Ketone **3** was then reacted with ammonia in methanol at 160 °C for 14 hours to provide a 5 : 1 mixture of **4** and 2-[^{14}C]-4-methoxy-2-ethylpyridine. This mixture was partially purified by chromatography on silica gel (43% yield of **4**) and then was treated with chlorine in sulfuric acid-water at -5 °C to provide a 54% yield of **5** after purification by chromatography on silica gel. Little

or no chlorination at the 5-position of **4** occurred as determined by capillary column GC-MS analysis of **4** and ^{1}H NMR analysis of the compound produced in an unlabeled run. The methoxypyridine side-product was found to be unreactive to the chlorination conditions and was easily separated by chromatography.

Aminopyridine **5** was then reacted with trimethylaluminum (4,5) in toluene at room temperature for 40 minutes and the resulting mixture treated with mixed anhydride **6** at 110 °C for 8 hours to provide, after chromatography on silica gel, a 63% yield of [^{14}C]-**1**. This coupling sequence was a major improvement over the classical methods utilized previously (1) to prepare compounds of this class (e.g., 1,3-dicyclohexylcarbodiimide promoted coupling of 4-aminopyridines and substituted phenylacetic acids or the coupling reactions of 4-aminopyridines and substituted phenylacetyl chlorides). The radiochemical purity of [^{14}C]-**1** was found to be 98% by reverse phase HPLC and normal phase TLC analyses. This value compared well with the chemical purity of [^{14}C]-**1** which was determined by reverse phase HPLC to be 98%. The specific activity of [^{14}C]-**1** was determined to be 21.10 mCi/mmol. The overall radiochemical yield for the 4-step sequence was 14% which compared very favorably with the 15% overall yield obtained in the unlabeled run.

EXPERIMENTAL

TLC analyses were conducted on either EM Reagents 5 x 20 cm Kieselgel silica gel 60 F254 plates or Whatman 5 x 20 cm KC18F plates. The plates were scanned on a Radiomatics RSTLC radioscanner. The data is listed as: plate used, solvent system, R_f, % radiochemical purity. The reverse phase HPLC analyses were conducted in a system consisting of a Water's 600E system controller, a Water's U6K injector and a Water's Novapak Phenyl column (4 μm, 5 x 100 mm in a Water's 8 x 10 RCM) connected in series with a Water's 990 Photodiode Array UV detector (250 nm) and a INUS B-Ram radioactivity monitor. The eluent (1 mL/min) was 40% acetonitrile / 60% water at time 0 and was changed with a linear gradient over 20 minutes to 100% acetonitrile and held there for 30 minutes. The GC analyses were conducted on a Hewlett Packard 5890 Series II Gas

Chromatograph equipped with a 5 meter HP-1 capillary column (0.53 mm, 2.65 μm film), a helium carrier gas at a flow of 20 mL per minute and a flame ionization detector. The oven temperature was held at 50 °C for 2 minutes and then was ramped to 250 °C at 20 °C/min and held there for an additional 10 minutes. The results are given in area percent. The mass spectra were recorded on a Finnigan 4615 mass spectrometer at 70 eV by direct exposure electron impact ionization. Total radioactivity measurements were made on a Packard 2500 TR Liquid Scintillation Analyzer using Ultima Gold Scintillation Cocktail. Methoxyenyne **2** was available from Fluka.

5-[^{14}C]-1-Methoxyhept-1-en-3-yn-5-one (3)

A solution of 74.6 mg (0.909 mmol) of 1-methoxy-1-buten-3-yne (Fluka, Lot # 321564/1 992, freshly distilled right before use, bp 112-117 °C @ 760 mm) in 1.5 mL of THF (SureSeal, Aldrich) was cooled to -78 °C and treated with 0.57 mL (0.91 mmol) of 1.60 M n-butyllithium in hexane. After stirring for 30 minutes at -78 °C, the solution was treated with 53.4 mg (0.455 mmol, 10.0 mCi, 22 mCi/mmol) of 1-[^{14}C]-N-methoxy-N-methylpropanamide (American Radiolabeled Chemicals, Inc., ARC-717, Lot # 930818) in 0.5 mL of THF (1.5 mL of rinse THF was used), stirred for 30 minutes, and then allowed to warm slowly to room temperature and stirred for one hour. The resulting yellow solution was then diluted with 7-8 mL of ether, cooled in ice, treated with 5.0 mL of 0.5 N aqueous HCl and warmed to room temperature with stirring. The aqueous phase was separated, extracted twice with ether (2 x 3 mL) and the combined organic phase was washed with saturated, aqueous, sodium bicarbonate solution (2 x 3 mL) and dried by passage through a short column of anhydrous sodium sulfate. The solvents were removed *in vacuo* to provide 66.6 mg (105% crude) of **3** as a brown oil. Because of the volatility of this compound no attempt was made to remove any residual solvents. This material was used directly in the next reaction.

2-[^{14}C]-4-Amino-2-ethylpyridine (4)

Crude **3** (66.6 mg) isolated above was dissolved in 2.0 mL of methanol, placed in a 40 mL Parr bomb with a magnetic stir bar, treated with 4.6 mL (9.1 mmol) of 2.0 M ammonia in methanol and

heated at 160-165 °C (195 psi) for 14 hours. After cooling to room temperature, the bomb was opened and the pale brown solution was concentrated *in vacuo* to 41.3 mg of a dark oil. This material was partially purified by chromatography on 0.75 g of silica gel (EM Science, Silica Gel 60, 230-400 mesh, eluents: 1 x 4 mL of methylene chloride, 3 x 3 mL of 80/20 methylene chloride/ methanol, 4 x 3 mL of methanol) to provide 8-3 mL fractions. Fractions 2-7 were combined and concentrated *in vacuo* to provide 29.6 mg (43%) of **4** as a brown oil. GC analysis indicated a 5:1 ratio of **4** to 2-[^{14}C]-4-methoxy-2-ethylpyridine (identified by GC-MS). This material was used without further purification in the next reaction.

2-[^{14}C]-4-Amino-3-chloro-2-ethylpyridine (5)

The 29.6 mg of crude **4** (ca. 0.2 mmol) isolated above was dissolved in 1.0 mL of 1:1 (v/v) sulfuric acid - water, cooled to -5 °C (salt-ice bath), stirred vigorously and treated dropwise with 0.2 mL (0.2 mmol) of 1.0 M chlorine in carbon tetrachloride. After stirring for 30 minutes, GC analysis indicated only 20-30% conversion so an additional 0.2 mL (0.2 mmol) of the 1.0 M chlorine solution was added at -5 °C. After stirring for another 30 minutes, GC analysis indicated a complete reaction. It appeared important to place the pipet (for chlorine addition) very near the reaction solution to minimize loss of chlorine due to volatilization from the carbon tetrachloride solution. The solution was made basic with 10-11 mL of 2 N sodium hydroxide (ice cooling) and then extracted with 4 x4 mL of methylene chloride. After analysis by GC, the organic phase was dried (sodium sulfate) and concentrated *in vacuo* to provide 26.5 mg of a brown oil. This material was purified by chromatography on silica gel (8.0 gm, 230-400 mesh, 22 x 40 mm bed, 96:4 (v/v) methylene chloride/methanol eluent) to provide 10 - 5 mL fractions. Fractions 4-7 were combined and concentrated to afford 16.3 mg (54%) of **5** as a brown solid.

4-(4-Chlorophenoxy)phenylacetic ethylformic anhydride (6, R = C(O)OEt)

A solution of 1.50 g (5.71 mmol) of 4-(4-chlorophenoxy)phenylacetic acid and 20 mL of methylene chloride was cooled to 0-5 °C and treated with 0.83 mL (6.00 mmol) of triethylamine and then 0.57

mL (6.00 mmol) of ethyl chloroformate. The resulting mixture was stirred at 0-5 °C for one hour, warmed to room temperature and stirred for 3.5 hours. The mixture was then diluted with 100 mL of ether and filtered. The filtrate was concentrated and the residue taken up in 10-15 mL of ether and filtered again. This filtrate was again concentrated under vacuum to provide 1.6 g (84%) of **6** (R = C(O)OEt) as a yellow oil. The crude product was used in the next reaction.

N-(2-Ethyl-3-chloro-4-pyridinyl-2-[^{14}C])-4-(4-chlorophenoxy)phenylacetamide ([^{14}C]-1)

The 16.3 mg (0.104 mmol) of **5** isolated above was transferred with the aid of methylene chloride through a pipet of anhydrous sodium sulfate to the reaction flask (15 mL rb flask). The solvent was evaporated with a stream of nitrogen gas and the residue was placed under high vacuum for 20 minutes. The residual solid **5** was dissolved in 0.5 mL of toluene (Aldrich, SureSeal) and treated at rt with 0.078 mL (0.16 mmol) of 2.0 M trimethylaluminum in toluene. After stirring at rt for 40 minutes, the solution was treated with 0.36 mL (0.16 mmol, 52 mg) of **6** (R = C(O)OEt) in toluene (145 mg/mL). The resulting solution was then heated at 100-110 °C for 8 hours. After cooling, the solution was treated with 2 drops of 1.0 M hydrochloric acid, stirred for a few minutes and then diluted with 5.0 mL of 9:1 ether/ethyl acetate (v/v) and 3.0 mL of 2.0 N sodium hydroxide. After stirring a few minutes (no emulsion), the aqueous phase was separated, extracted with 9:1 ether / ethyl acetate (2 x 5 mL) and the combined organic layers were washed with 5 mL of saturated, aqueous, sodium chloride solution. After drying (sodium sulfate), the organic solution was concentrated *in vacuo* to afford 65 mg of a light brown oil. This material was purified by chromatography on silica gel (17 g, 230-400 mesh, 25 mm x 75 mm, 55:45 (v/v) hexane/ethyl acetate eluent). A 50 mL fraction was taken and then 12 - 5 mL fractions were collected. Fractions 5-11 were combined and concentrated, and the resulting oil was dissolved in acetone, filtered thru a plug of glass wool into a tared flask, rotovaped to dryness, and then placed under a high vacuum until a constant weight was obtained to afford 26.2 mg (63%) of [^{14}C]-**1** as a colorless oil. Analysis indicated 1.34 mCi of [^{14}C]-**1** at a specific activity of 21.10 mCi/mmol: TLC analyses (1) SiO_2, 50/50 hexane/ethyl acetate, R_f 0.28, 97.9%, (2) SiO_2, 60/40 hexane/acetone, R_f 0.36, 97.0%,

(3) SiO_2, 98/2 chloroform/methanol, R_f 0.21, 98.1%, and (4) KC18F, 95/5 acetonitrile /water, R_f 0.42, 97.5%; HPLC analysis 21.44 min, 97.9%; MS 400, 402 & 404 (M^+), 365, 367 & 369 (M^+-Cl). The tracer was compared with an unlabeled standard of [^{14}C]-**1** during the above analyses and afforded identical results.

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