SYNTHESES OF 2-([^{14}C]METHYL)FURAN AND 4-OXO[5-^{14}C]-2-PENTENAL

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SUMMARY

A simple one-step syntheses of 2-([^{14}C]-methyl)furan and 4-oxo-[5-^{14}C]-2-pentenal have been described. Carbon-14 labeled 2-methylfuran was synthesized by treatment of 2-furyl lithium with ^{14}C-methyl iodide, and ^{14}C-acetylacrolein was obtained by peracid oxidation of labeled 2-methylfuran.

Key Words - 2-([^{14}C]methyl)furan, 4-oxo[5-^{14}C]-2-pentenal, peracid oxidation

INTRODUCTION

The synthesis of tritium labeled 2-methylfuran (2-MF) and 3-methylfuran (3-MF) were earlier reported from this laboratory (1). 2-Methylfuran and 3-MF are capable of producing cellular necrosis of liver, lungs or kidney, depending upon the particular animal species tested. These methylfurans are metabolically activated to reactive electrophilic intermediates which bind covalently to tissue macromolecules (2,3). We recently reported the isolation of acetylacrolein (4-oxo-2-pentenal) as the reactive metabolite of 2-MF that binds to microsomal proteins in vitro (4,5). Tritium, labeled in the alkyl moiety of 2-MF, would not be metabolically stable, more so after metabolic transformation to acetylacrolein (AA). It was hence necessary to synthesize carbon-14 labeled 2-MF for use in metabolic studies in biological systems. Also, in order to explore the possibility that acetylacrolein was the ultimate reactive metabolite that was bound to macromolecules, it was necessary to devise a method of synthesis of ^{14}C-AA.

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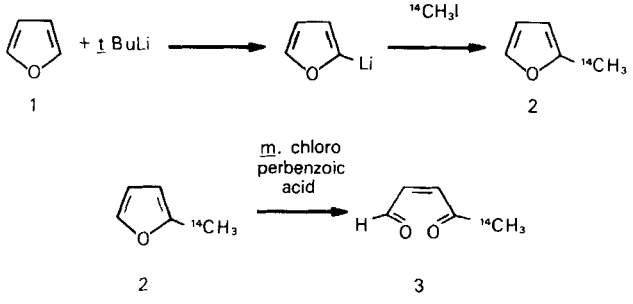
Carbon-14 labeled 2-MF was synthesized by reaction of 2-furyl lithium with [^{14}C]-methyl iodide, and m-chloroperbenzoic acid oxidation of ^{14}C-2-MF yielded ^{14}C-AA (Scheme 1).



RESULTS AND DISCUSSION

The synthesis of 2-([^{14}C]methyl)furan described here is a facile one-step method, alleviating the requirement for the unstable and explosive 2-furfuryl chloride used in the synthesis of tritium labeled 2-MF. The specific activity of 2-[^{14}C]-methylfuran is considerably diluted by the addition of unlabeled methyl iodide following the addition of [^{14}C] methyl iodide. The specific activity of 2-([^{14}C]methyl)furan can be increased substantially by using only [^{14}C]-methyl iodide, undiluted with any unlabeled methyl iodide. The radiochemical yield of 2-([^{14}C]methyl)furan may be increased by minimizing the loss of [^{14}C]-methyl iodide during transfer of the reagent. The method is also adaptable to large scale preparation.

Acetylacrolein (AA) is an extremely reactive compound, and the carbon-14 labeled AA had to be purified by flash chromatography where the contact time of AA with silica gel was minimal. The product obtained by purification of AA by flash chromatography was pure cis-AA as indicated by NMR spectrum; capable of detecting up to 5%, the presence of the trans isomer. During HPLC analysis of the purified cis-AA, partial isomerization of cis-AA to trans-AA took place. This was also apparent from the UV spectra of the two compounds. While the cis isomer has an absorption maximum at 270 nm, the trans isomer absorbs at a higher wavelength. The isomerization of cis-AA to trans-AA in acidic methanol has been reported earlier (7). The trans isomer easily formed the dimethylacetal,

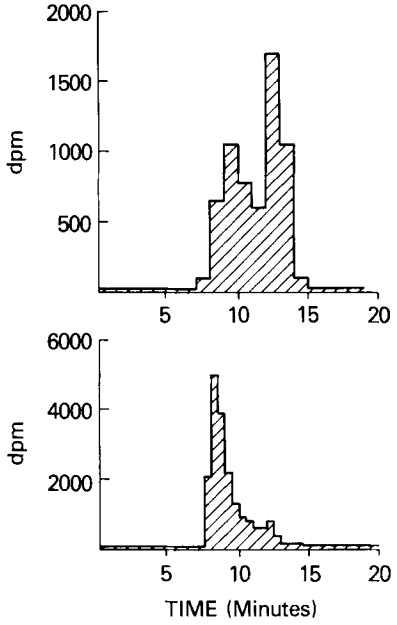


Figure 1. Radiochromatogram of high pressure liquid chromatographic analysis of ^{14}C-acetylacrolein (a) before and (b) after treatment with methanol (see text for details).

and no absorption maximum above 220 nm was observed (7). Thus, when cis-^{14}C-AA was treated with methanol for 6 hr at 4°C and then analyzed on HPLC (Fig. 1), the proportion of the trans isomer increased as indicated by the increase in the amount of label in the second peak of the upper chromatogram (Fig. 1) demonstrating the increased formation of the dimethylacetal of trans-AA. The ultraviolet spectra of this compound shows no absorption maxima above 220 nm (7). Although after the initial purification of ^{14}C-AA by flash chromatography, exclusively cis-^{14}C-AA was obtained, it underwent isomerization during the determination of its radiochemical purity by HPLC. However, taking into account the amount of ^{14}C-AA present in both the cis form (87% of total) and the trans form (13% of total), after HPLC analysis the radiochemical purity was greater than 96%.

Both ^{14}C-AA and ^{14}C-2-MF are sensitive to air and subject to photodecomposition. Carbon-14 labeled 2-MF should be stored in the dark at -70°C and be purified by preparative gas chromatography immediately prior to use in biological experiments, and ^{14}C-AA should be synthesized and purified immediately before use.

EXPERIMENTAL

Authentic 2-MF, furan, t-butyl lithium in pentane and m-chlorperbenzoic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI) and carbon-14 labeled methyl iodide (sp. activity 30 mCi/mmole) from ICN Chemical and Radioisotope Division (Irvine, CA). Silica gel 60 (200-400 mesh) was supplied by E. Merck, Germany. All solvents were distilled prior to use.

Preparative gas chromatography was performed on a Varian Aerograph Model 700 (Varian Associates, Palo Alto, CA), equipped with a thermal conductivity detector and a 25 m aluminum column (0.7 cm i.d.), packed with 10% OV-101 on 100/120 Gas-Chrom Q (Applied Science, State College, PA). Carrier gas (helium) was maintained at a flow rate of 120 ml/min. The column oven temperature was maintained at 80°C, while the injector and detector temperature were kept constant at 150°C.

HPLC analysis was carried out on a Waters Associates (Milford, MA ALC/6PC204) liquid chromatograph equipped with a Kratos Model 773 variable wavelength detector using a Waters Associates μ-Bondapak C_{18} column (0.94 x 50 cm) with 5% acetonitrile/water as mobile phase.

Proton NMR spectra were recorded at room temperature on a JEOL-60 FX spectrometer in deutrated chloroform with tetramethylsilane as internal standard. Gas chromatography mass-spectral analysis was performed on a Varian MAT 44 spectrometer. The GC separations were accomplished on a glass column containing 3% OV-17, operated over a temperature gradient of 30°-100°C (5°C/min), using helium as carrier gas. Mass spectroscopy was performed in the electron-impact mode. Ultraviolet spectra were recorded on an HP-8450 spectrophotometer (Hewlett Packard, Palo Alto, CA).

2-([^{14}C]methyl)furan: The synthesis was an adaptation of the procedure described by Benkeser and Currie for the synthesis of 2-furyl trimethylsilane

(8). Furan, 1, 0.122 g (1.8 mmol) in 5 ml of dry ether was placed in a three-neck flask under an atmosphere of dry nitrogen and a solution of t-butyl lithium in pentane (0.122 g, 1.8 mmol) was added dropwise with stirring at room temperature. The reaction mixture was stirred for 2 hr and [^{14}C]-methyl iodide 0.004 g (30 mCi/mmol) was added. After stirring for 1 hr, methyl iodide 0.13 g (0.9 mmol) was added and the stirring continued for 3 hr. The reaction was stopped by the addition of 1 ml of 0.05 N HCl. The organic layer was separated, washed with water and dried over anhydrous sodium sulfate.

Aliquots of the ether solution were withdrawn using a 250 µl glass syringe (Pressure-Lok Gas Syringe, Precision Sampling Corp., Baton Rouge, LA) and injected onto a preparative gas chromatography column (GC). Carbon-14 labeled 2-MF was trapped in a glass vessel immersed in liquid nitrogen. The product was examined by mass spectrometry and found to exhibit exactly the same mass spectrum as pure, authentic 2-MF [yield 24.6 mg, (yield 33%) sp. activity 0.1 mCi/mmol, radiochemical yield 9%). The carbon-14 labeled 2-MF was diluted with pure, authentic, nonradioactive 2-MF and injected on to the preparative GC. The effluent was collected every two minutes in a glass trap under liquid nitrogen. The condensate was flushed out with 3 ml of methanol and counted in a liquid scintillation counter after the addition of 10 ml of scintillation liquid. The radiochromatographic purity was > 98%.

^{14}C-Acetylacrolein 4: m-Chloroperbenzoic acid (210 mg, 1.03 mmol) was dissolved in 2 ml of dichloromethane in a 5 ml reactivial equipped with a teflon septum and immersed in an ice-bath at 4°C. Carbon-14 labeled 2-MF 3 [98.4 mg (1.2 mmol) sp. activity 1.7 µCi/mmol) was injected slowly through the teflon septum. After being stirred at 4°C for 15 min, the reaction was stopped by the addition of 1 ml of saturated sodium bicarbonate solution, vortexed and the aqueous layer removed. The organic layer was washed once again with 1 ml saturated sodium bicarbonate, followed by water and dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo and the crude ^{14}C-AA was purified by flash chromatography over a column of silica

gel using 25% ethylacetate-hexane as mobile phase. Fractions were collected every 15 sec and an aliquot was monitored for radioactivity by liquid scintillation counting. Another aliquot of each fraction was spotted on a silica gel coated TLC plate along with authentic acetylacrolein (solvent 1:3 ethylacetate-hexane). Fractions containing pure ^{14}C-AA 4 were combined and solvent evaporated under vacuum (yield 50 mg, 42.5% sp. activity 1.7 μCi/mmol).

Radiochemical purity was established by analyzing an aliquot of purified ^{14}C-AA by HPLC on a μ C_{18} Bondapak column using 5% acetonitrile/water as mobile phase at a flow rate of 2 ml/min. Fractions were collected every 0.5 min and the radioactivity determined by liquid scintillation counting (Fig. 1). The radiochemical purity of ^{14}C-AA was > 96%.

The flash chromatographically purified ^{14}C-AA was examined by mass spectrometry and NMR, and found to be identical with authentic sample. m/e (relative abundance %) - 98 (5.69, M+), 89 (19.63), 55 (66.04), 43 (100), 42 (59.96).

NMR - δ ppm - 10.2 (d, 1H, J=7Hz), 6.96 (d, 1H, J=12 Hz), 6.15 (q, 1H, J=12.7 Hz) 2.38 (3H, s).

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