

AN IMPROVED SYNTHESIS OF [1-¹⁴C]-LABELLED 2-BUTYNYDIOIC ACID,
SUCCINIC ACID AND SUCCINYL CHLORIDE

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SUMMARY

The reaction of [¹⁴C]CO₂, generated from [¹⁴C]BaCO₃, with dilithio propiolate (2) at -78°C afforded [1-¹⁴C]-2-butyndioic acid (3). Reduction of 3 with platinum oxide and hydrogen gas gave [1-¹⁴C]-succinic acid (4) in 74% overall radiochemical yield from [¹⁴C]BaCO₃ with a specific activity of 55.4 mCi/mmol (2.05 GBq/mmol). Reaction of 4 with dichloromethyl methyl ether in the presence of zinc chloride yielded [1-¹⁴C]-succinyl chloride (5, 97.5% radiochemical yield).

Key words: Dilithio propiolate, electrophilic carboxylation, [1-¹⁴C]-labelled 2-butyndioic acid, succinic acid and succinyl chloride

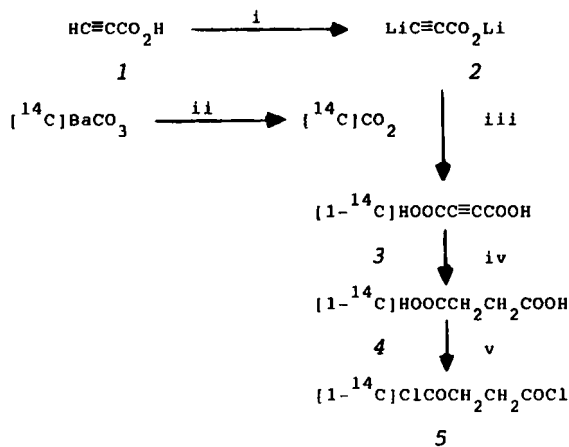
INTRODUCTION

In the course of investigations in biological and metabolic studies it became necessary to prepare high specific activity [1-¹⁴C]succinic acid. Although a number of methods have been reported (1-4) for the synthesis of isotopic succinic acid, each method suffers from one or more disadvantages which may include: i, low specific activity product (1-3); ii, low radiochemical yield (3); iii, a long synthetic reaction sequence (4); iv, tedious purification procedures (4), and v, expensive [¹⁴C]-reagents (1,2,5). We now report a facile procedure for the synthesis of high specific activity [1-¹⁴C]-labelled 2-butyndioic acid (3), succinic acid (4) and succinyl chloride (5) in high radiochemical yield which circumvents all of the inherent disadvantages listed above (6).

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RESULTS AND DISCUSSION

In selecting a synthetic procedure for synthesis of the required $[1-^{14}\text{C}]$ -succinic acid (4) we decided to investigate the reaction of dilithio propiolate (2), generated *in situ* by treatment of commercially available propiolic acid (1) with 1.97 equivalents tert-butyllithium at -78°C in anhydrous tetrahydrofuran, with $[^{14}\text{C}]\text{CO}_2$. It was expected that the sterically hindered base tert-butyllithium would selectively abstract the two acidic hydrogens present in 1 at low temperature rather than reacting as a nucleophile in a Michael type reaction involving addition to the alkyne bond of 1. $[^{14}\text{C}]\text{CO}_2$ was prepared by the reaction of concentrated sulfuric acid with commercial $[^{14}\text{C}]\text{BaCO}_3$ (56.4 mCi/mmol, 2.08 GBq/mmol) and was manipulated by vacuum and cryogenic techniques using the apparatus depicted in Fig. 1. Thus, reaction of 2 with $[^{14}\text{C}]\text{CO}_2$, as described above, afforded $[1-^{14}\text{C}]$ -2-butyne-1,4-dioic acid (3, 77.6% chemical yield, 38.2 mCi, 1.41 GBq,



Reagents: i, tert-BuLi, THF, -78°C ; ii, H_2SO_4 ; iii, $[^{14}\text{C}]\text{CO}_2$;

iv, H_2/PtO_2 ; v, ZnCl_2 , $90-100^\circ\text{C}$ (oil bath temperature)

76.3% radiochemical yield, specific activity 55.43 mCi/mmol). The product $[1-^{14}\text{C}]$ -3 did not require purification since the only contaminant present was unreacted propiolic acid (1) which was readily removed *in vacuo* (40°C , 0.5 mm Hg). The quantitative reduction of $[1-^{14}\text{C}]$ -3 with platinum oxide and hydrogen gas at 15 psi yielded $[1-^{14}\text{C}]$ -succinic acid (4, 97.3% isolated chemical and radiochemical yield) with a radiochemical purity of 99.3%.

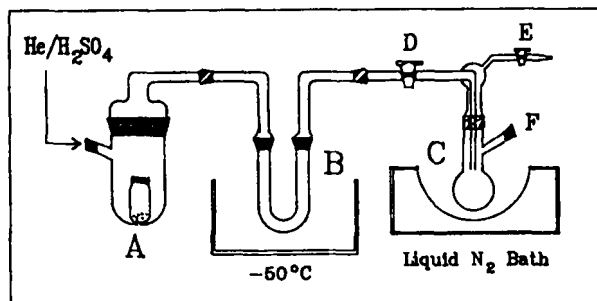


Fig 1. Apparatus used for the generation of $[^{14}\text{C}]\text{CO}_2$ and the synthesis of $[1-^{14}\text{C}]\text{-2-butynedioic acid}$

Treatment of $[1-^{14}\text{C}]\text{-4}$ with the chloride-transfer reagent dichloromethyl methyl ether (DCME) in the presence of anhydrous zinc chloride, to prevent the formation of succinic anhydride, using the procedure of Rieche et al (7) afforded $[1-^{14}\text{C}]\text{succinyl chloride (5)}$ in 97.5% radiochemical yield. Excess DCME, and methyl formate formed during the reaction, were removed *in vacuo* (40°C , 0.5 mm Hg).

SUMMARY

High specific activity (55.4 mCi/mmol) $[1-^{14}\text{C}]\text{labelled 2-butynedioic acid (3)}$, succinic acid (4) and succinyl chloride (5) were synthesized in high radiochemical yield in two, three and four step reaction sequences, respectively. The radiochemical purity of compounds 3-5 was sufficiently high that further purification was not required.

EXPERIMENTAL

All solvents were glass distilled before use and, where anhydrous solvents were required, these were dried by standard methods. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Whatman MK6F microslides using chloroform:methanol:water (65:30:5 v/v/v) as development solvent. ^1H NMR spectra were acquired using a Bruker AM-300 300 MHz spectrometer. Infrared spectra were recorded on a Nicolet 5DX FT spectrometer. Radioactive counting was performed using Aquasol-2 fluor (New England Nuclear) in a Beckman LS 9000 liquid scintillation counter. The radioactive constituents of reaction mixtures were analyzed by fractionating a

developed TLC plate, analyzing the fractions by liquid scintillation counting and plotting histograms of the resulting data (combined TLC-LSC). Propiolic acid (1), purchased from the Aldrich Chemical Co., was distilled prior to use. Dichloromethyl methyl ether (DCME) was purchased from Terrochem Laboratories, Edmonton, Canada. $[^{14}\text{C}]\text{BaCO}_3$ was purchased from Atomic Energy of Canada Ltd. The specialized glassware, illustrated in Fig. 1, was fabricated by the University of Alberta Technical Services Glass Shop.

Generation of $[^{14}\text{C}]$ carbon dioxide

The apparatus, depicted in Fig. 1 for the generation of $[^{14}\text{C}]\text{CO}_2$, was dried in an oven and assembled hot. After cooling using a flow of dry helium gas, the vial containing $[^{14}\text{C}]\text{BaCO}_3$ (0.177 g, 0.888 mmol, 56.4 mCi/mmol, 2.08 GBq/mmol) was carefully inserted into generator flask A. Trap B was maintained at -50°C (to remove traces of water and acid) and trap C was maintained at -196°C (liquid-nitrogen bath). The entire apparatus was evacuated and $[^{14}\text{C}]\text{CO}_2$ was generated by addition of concentrated sulfuric acid (10 mL) dropwise to $[^{14}\text{C}]\text{BaCO}_3$ in the generator flask A via a syringe. The $[^{14}\text{C}]\text{CO}_2$ generated was collected in trap C (reaction flask). When the evolution of $[^{14}\text{C}]\text{CO}_2$ had ceased the generator flask A was warmed to 50°C to transfer residual $[^{14}\text{C}]\text{CO}_2$, with a slow flow of dry helium gas, to trap C. Heating at 50°C was continued for 30 min after which flask A was allowed to cool to 25°C . Trap C containing $[^{14}\text{C}]\text{CO}_2$ was disconnected from the remainder of the apparatus, the remaining system was evacuated, stopcock E was closed and flask C was maintained at -196°C prior to subsequent use.

Dilithio propiolate (2)

A solution of tert-butyllithium (1.89 mmol) in hexane (0.9 mL of a 2.1 M solution) was added dropwise with stirring to a solution of propiolic acid (1, 67.5 mg, 0.96 mmol) in dry tetrahydrofuran (20 mL) at -78°C in a 50 mL round bottom flask equipped with a side arm, under an atmosphere of dry nitrogen. This reaction mixture was maintained at -78°C for 15 min with stirring prior to use.

$[1-^{14}\text{C}]-2\text{-Butynedioic acid (3)}$

The above solution of dilithio propiolate (2) was transferred to trap C using a double ended needle via outlet F. The contents of trap C were then allowed to

warm to -78°C and stirring was continued for 2 hr. The resulting pale yellow-white suspension which formed was stirred while the temperature was allowed to warm slowly to 0°C . A solution of 10% sulfuric acid (10 mL) was added slowly and stirring was continued for 30 min at 0°C . After removing most of the tetrahydrofuran *in vacuo*, the residue was extracted with diethyl ether (5 x 5 mL). The combined ether extracts were back extracted with water (1 mL), the ether fraction was dried (anhydrous Na_2SO_4) and the solvent was removed *in vacuo* to yield **3** (80 mg, 0.69 mmol, 77.6% chemical yield, mp $178\text{--}180^{\circ}\text{C}$, lit (8) mp 179°C). The product **3** was dissolved in 95% EtOH and the volume was adjusted to 50 mL. Radioactivity measurements, using liquid scintillation counting, indicated that the total activity present was 38.2 mCi (1.41 GBq), that the specific activity was 55.43 mCi/mmol, and that the radiochemical yield from $[^{14}\text{C}]\text{BaCO}_3$ was 76.3%. The product **3**, which was analyzed by TLC, exhibited a single spot having R_f 0.4 that was identical to that of an authentic unlabelled sample of **3**. The TLC plate was divided into 10 equal sections and the silica gel sections were scraped directly into individual scintillation vials for radioactivity measurements. The following results were obtained (section, number of counts, % of total activity applied to the TLC plate): 1, 0, 0.0; 2, 10, 0.03; 3, 13, 0.04; 4, 5457, 18.0; 5, 19999, 66.1; 6, 3584, 11.8; 7, 978, 3.2; 8, 182, 0.60; 9, 7, 0.02; 10, 14, 0.04. Those sections of the plate corresponding to the R_f for **3** (sections 4-7) accounted for 99.1% of the total activity applied to the TLC plate, viz 99.1% radiochemical purity.

$[1\text{-}^{14}]\text{Succinic acid (4)}$

A mixture of **3** (37 mCi total activity) and platinum oxide (20 mg) in 95% ethanol (10 mL) was allowed to stir under one atmosphere of hydrogen gas at 25°C for 3 hr. The product, obtained after filtration and removal of the solvent *in vacuo*, gave **4** (78 mg, 0.649 mmol, 97.3% chemical and radiochemical yield, 36.0 mCi (1.33 GBq) total activity, specific activity 55.4 mCi/mmol, mp $185\text{--}187^{\circ}\text{C}$, lit (3) mp 185°C). Product **4** exhibited a ^1H NMR spectrum (deuterium oxide) which was identical to that of an authentic unlabelled sample of succinic acid (δ 2.64, singlet). The product **4**, which was analyzed by TLC using the methodology described above for **3**, exhibited one major spot at R_f 0.25 which was identical to that of an authentic unlabelled sample of succinic acid. TLC radioactivity analysis (section, number of counts,

% of total activity applied to the TLC plate) indicated the following results for the 10 sections obtained from the TLC plate as described above: 1, 15, 0.06; 2, 161, 0.07; 3, 240, 1.10; 4, 940, 4.35; 5, 8042, 37.28; 6, 9718, 45.0; 7, 1989, 9.20; 8, 347, 1.60; 9, 82, 0.38; 10, 16, 0.07. Those sections of the plate corresponding to the R_f for 4 (sections 4-8) accounted for 99.3% of the total activity applied to the TLC plate, viz 99.3% radiochemical purity.

[1-¹⁴C]Succinyl chloride (5)

The procedure used was identical to that described by Rieche et al (7) for the synthesis of unlabelled 5. Anhydrous zinc chloride (10 mg) was added to a solution of [1-¹⁴C]-4 (70 mg, 32.3 mCi total activity) in dry dichloromethyl methyl ether (2 mL) and the reaction mixture was allowed to stir at 25°C for 10 min prior to heating in an oil bath at 90-100°C for 1 hr. The reaction mixture was allowed to cool to 25°C, and the excess dichloromethyl methyl ether and resulting methyl formate formed during the reaction were removed *in vacuo*. The product 5 exhibited an infrared spectrum (benzene solution) identical to that of an unlabelled authentic sample of 5 [1789 cm⁻¹ (COCl)]. The spectrum did not exhibit a peak at 1867 cm⁻¹ which is present in the infrared spectrum of succinic anhydride. Dissolution of 5 in dichloromethane (10 mL) and quantitation of radioactivity by liquid scintillation counting indicated that the product 5 contained 31.5 mCi activity and that the radiochemical yield was 97.5%.

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