SYNTHESIS OF MALONDIALDEHYDE-1,2,3-$^{14}C_3$ VIA ETHYL VINYL-[1,2-$^{14}C_2$] ETHER

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SUMMARY

The synthesis of 1,1,3,3-tetraethoxypropane-1,2,3-$^{14}C_3$ from uniformly labeled paraldehyde is described. The synthesis involves three steps and proceeds in an overall yield of 25%. The final product is greater than 95% radiochemically pure and it is stable indefinitely when stored as thoroughly degassed benzene solutions. Hydrolysis of radiolabeled tetraethoxypropane occurs in moderate yields to form malondialdehyde-1,2,3-$^{14}C_3$(1,3 propanedial-1,2,3-$^{14}C_3$) which is isolated and stored as the sodium salt. One of the intermediates in the synthesis of tetraethoxypropane is ethyl vinyl-[1,2-$^{14}C_2$] ether, which is isolable in 50% overall yield from paraldehyde. The widespread utilization of ethyl vinyl ether in organic synthesis suggests that the radiolabeled material should provide an entry to a large number of specifically [^{14}C] labeled compounds.

Key Words: Malondialdehyde-1,2,3-$^{14}C_3$, Ethyl Vinyl-[1,2-$^{14}C_2$] Ether.

INTRODUCTION

Mammalian tissues produce substantial quantities of malondialdehyde, 1, during prostaglandin biosynthesis[1-3] and lipid peroxidation[4]. It is a reactive electrophile and covalently attaches to intracellular nucleophiles such as proteins[5] and nucleic acids[6,7]. Furthermore, malonaldehyde is mutagenic in Salmonella typhimurium[8] and carcinogenic in female mice[9] suggesting that it might be an important agent in cellular degeneration and dysfunction. Remarkably little is known about the metabolic disposition of malondialdehyde because of the unavailability of radiolabeled material for tracer studies. Recently, Summerfield and Tappel[10] have reported that malondialdehyde-1,3,-$^{14}C_2$ can be biosynthesized by the action of alcohol dehydrogenase on 1,3-propanediol-1,3-$^{14}C_2$. Neither the specific activity nor the radiochemical purity of the final product

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was reported. This paper describes the chemical synthesis of malondialdehyde-1,2,3-$^{14}C_3$ from commercially available paraldehyde-U-^{14}C. An intermediate in the synthesis is ethyl vinyl-[1,2-$^{14}C_2$] ether 5. This is the first reported preparation of this compound labeled with [^{14}C] in the vinyl group. The widespread use of ethyl vinyl ether in organic syntheses suggests that 5 should be a valuable intermediate for the preparation of many other specifically [^{14}C]-labeled compounds.

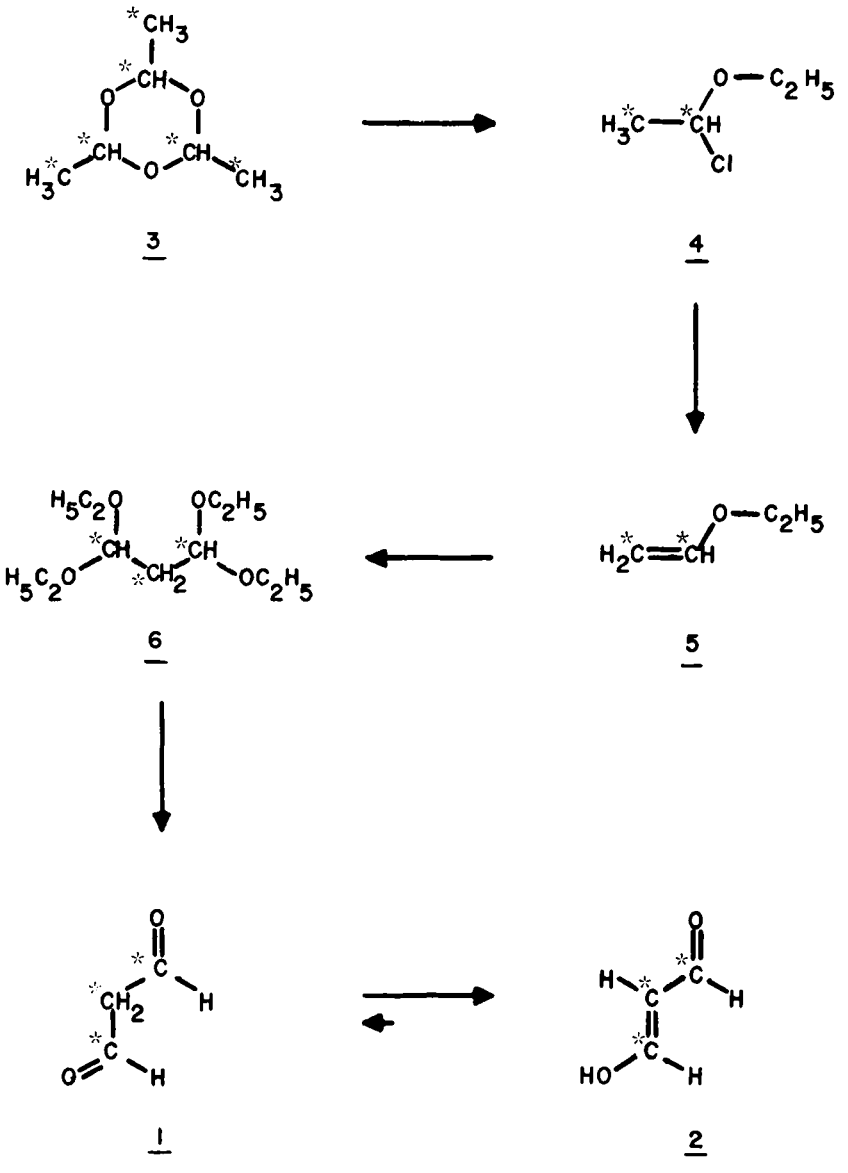
RESULTS AND DISCUSSION

Scheme I outlines the synthetic pathway employed. Although only two carbons are actually labeled, the symmetry of 6 and 1 effectively labels all three carbons. The specific activity of carbon 2 is twice that of carbons 1 and 3. The maximum specific activity of 1 is limited by the quantity of carrier which must be added for efficient handling of 4 and 5. The highest value which we have attained is 100 μCi/mmol.

The major difficulty in the synthesis involves the preparation and dehydrohalogenation of the chloro ether 4[11]. This compound is unstable above 0° and must be handled carefully to avoid decomposition. Pyridine catalyzed elimination of HCl from 4 proceeds in only 15% yield (lit ~ 45%)[12]. A survey of a number of common bases revealed that the conversion of 4 to 5 is consistently affected in yields greater than 70% by 2,4,6-collidine.[13] This is a significant improvement over current literature procedures.

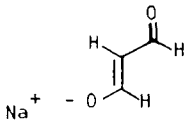
Addition of triethyl orthoformate to 5 proceeds in 72% yield to 1,1,3,3-tetraethoxypropane-1,2,3-$^{14}C_3$, 6.[14] The isolated product gives the expected spectral and combustion analyses. The radiochemical purity of 6 is in excess of 95% as determined by thin layer chromatography and gas chromatography. Solutions of 6 in benzene undergo appreciable degradation when stored at -20°. The rate of degradation is significantly reduced by repeated freeze-pump-thaw degassing prior to storage. Solutions of 6 prepared in this fashion can be stored for one year without appreciable decomposition.

Scheme I



* Indicates ^{14}C

The standard method of hydrolysis of 6 to malondialdehyde is described by Protopopova and Skoldinov.[15] This involves treatment with dilute hydrochloric acid at moderate temperatures, followed by neutralization with sodium hydroxide, and precipitation of the sodium salt of malondialdehyde (pKa_1 = 4.46[16]) with acetone. Conversion of 1 to its sodium salt is necessitated by the instability of 1 as the free acid.[17] The hydrolysis of tetraalkoxypropanes to 1 according to the standard method generates substantial amounts of highly colored impurities[18] and modifications of the hydrolytic procedure are described.[19] These modifications produce purer sodium malonaldehydate 7 but the yields following acetone precipitation are somewhat reduced. In fact, we find that the hydrolysis, neutralization, precipitation sequence is inefficient for the preparation of small amounts of 7. A superior method involves chromatography on Sephadex LH-20 followed by lyophilization for the isolation of 7 from neutralized hydrolysis mixtures. Since NaCl cochromatographs with 7 on Sephadex LH-20, Dowex 50 is employed instead of HCl as the acid catalyst for the hydrolysis of 6. The isolated yields of 7 are approximately 40% with a purity of 95%. The column procedure can readily be used for the isolation of 7 in amounts ranging from 1mg to 1 g.



7

EXPERIMENTAL

Uniformly [^{14}C]-labeled paraldehyde was obtained from New England Nuclear. Unlabeled paraldehyde and triethyl orthoformate were obtained from commercial

sources and purified by vacuum distillation immediately prior to use. 2,4,6-collidine was purified by vacuum distillation from barium oxide and stored over molecular sieves. Silica gel for column chromatography was from Baker (60-200 mesh) and Sephadex LH-20 was from Pharmacia. Radioactive compounds on TLC plates were detected using a Berthold LB-2760 radiochromatogram scanner. Radiogas chromatography was performed with a Varian 3700 interfaced to a Panax radiogas detector.

α-Chloroethyl ethyl ether 4. 1.3 g (0.01 mol) of freshly distilled paraldehyde containing 3 mCi of uniformly [^{14}C]-labeled paraldehyde and 1.38 g absolute ethanol were cooled in an ice bath and bubbled with HCl for one hour resulting in the formation of two layers. The upper layer was transferred to a chilled test tube containing a small amount of anhydrous calcium chloride and bubbled with dry nitrogen to remove excess HCl. After one hour the liquid (2.1 g, 66%) was drawn off and used immediately for the next step.

Ethyl vinyl ether 5. 2.1 g of 4 (0.019 mol) was added dropwise to 6 g (0.05 mol) of 2,4,6-collidine at ambient temperature and a white precipitate immediately formed. After the addition was complete the addition funnel was replaced with a short path distillation apparatus and the mixture heated to 190°. 5 distilled and was trapped in a receiver immersed in dry ice-isopropanol. (0.95 g, 70%)

1,1,3,3-Tetraethoxypropane 6. 0.95 g of 5 (0.013 mol) was added to 6.1 g triethyl orthoformate (0.038 mol) containing 20 μL of boron triflouride etherate and the mixture stirred at ambient temperature for 30 min. The reaction was quenched by the addition of sodium bicarbonate. After filtration, the excess triethyl orthoformate was removed by short path distillation and then 6 was vacuum distilled (45°, 0.3 Torr). Additional 6 was obtained by chromatographing the pot residue on a silica gel column with 9/1-hexane/ether. The total yield of 6 from 5 was 1.6 g (55%) corresponding to an overall yield of 25% from 3. NMR, mass

spectral, and combustion analysis were identical to those of an authentic standard. The radiochemical purity was determined following TLC (silica gel, hexane/ether-75/25) and GC (3'-3% OV-25, 40 ml Ar/min, 45-200° at 10°/min) and was found to be in excess of 95% by both methods.

Sodium malonaldehydate 7. 1.1 g of 6 (0.005 mol), 3.3 g Dowex 50 (0.005 mol H^+), and 10 g H_2O were gently agitated in a shaking water bath at ambient temperature until dissolution was complete (~20 min). After 5 additional minutes the Dowex was filtered off and the solution carefully titrated to pH 7 with 5 and 1 N NaOH. The sample was concentrated by lyophilization, applied to a column of Sephadex LH-20 (2 x 40 cm) and eluted with twice distilled H_2O. Samples contained in smaller volumes can be applied directly onto the column without lyophilization. The major radioactive zone that eluted from the column contained 7. Fractions containing this material were lyophilized leaving a highly electrostatic white powder corresponding to a 40% yield. The NMR spectrum (D_2O) exhibited a triplet at 5.13 δ (J = 10.0 Hz) and a doublet at 8.46 δ (J = 10 Hz).[20] The purity of this material was in excess of 95% by NMR. It can be further purified by repeated LH-20 chromatography or by recrystallization from water/acetone. In four separate preparations, the radiochemical purity of 7 varied from 91 to 98%.

ACKNOWLEDGEMENT

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