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SYNTHESIS OF METHYL ARACHIDONATE-17,17,18,18-d_4

Richard Adlof
Northern Regional Research Center, Agricultural Research Service,
U.S. Department of Agriculture, Peoria, Illinois 61604

SUMMARY

Methyl arachidonate (5c,8c,11c,14c-20:4) 17,17,18,18-d_4 was prepared for use in both *in vivo* and *in vitro* metabolism studies. The deuterium atoms were incorporated by deuterium gas and Wilkinson's catalyst. The tetraacetylenic acid precursor (5a,8a,11a,14a-20:4) 17,17,18,18-d_4 was synthesized by a series of Grignard coupling reactions and reduced by Lindlar catalyst and hydrogen gas to arachidonic acid-d_4. The methyl ester was prepared by diazomethane and purified by a combination of reverse phase and silver resin chromatography. The deuterated methyl arachidonate was prepared in an overall yield of ca. 11% and with an isotopic purity of >91%. The deuterium-labelled tetracetylenic acid precursor and the methyl arachidonate-d_4 were characterized by ^{13}C-NMR.

Key Words: Deuterium, fatty acid, arachidonate, synthesis, ^{13}C-NMR

INTRODUCTION

Methyl arachidonate (methyl 5c,8c,11c,14c-eicosatetraenoate) -17,17,18,18-d_4 was synthesized as part of a study to monitor fatty acid metabolism in humans. Arachidonic acid, one of the essential fatty acids, has been recognized as an important precursor to prostaglandins and an important component of phospholipids as well as being implicated in atherosclerosis (1,2). Deuterium-labelled arachidonate was required in studies to elucidate human metabolic pathways. Other researchers have synthesized methyl arachidonate with deuterium atoms on the double-bonds (5,6,8,9,11,12,14,15-d_8) (3-9). However, isotopic purities were only in the range of 55-60% d_8.

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Arachidonic acid-20,20,20-d_3 has also been prepared (10), but in an overall yield of < 2%. A synthetic sequence recently developed by our group (11) was modified for preparation of multi-gram quantities of deuterium-labelled arachidonate, in an overall yield of > 11% and of > 91% isotopic purity. The deuterium atoms were located on the 17- and 18-carbon atoms to minimize potential isotope effects.

EXPERIMENTAL

Instruments. Isotopic purity and deuterium distribution were determined on a Finnigan Model 4500 gas chromatograph (GC)/EI-CI Mass Spectrometer (MS) equipped with a 30 m Supelcowax 10 capillary column (.32 mm x 0.5 micron film thickness). Helium was used as carrier gas. The GC was programmed from 165° C to 265° C at 5° C/min. Cis and trans isomers were analyzed with a Packard Model 428 GC equipped with a 100 m SP2560 capillary column (helium carrier gas; FID). Synthetic intermediates were analyzed on a 10 ft x 3/8 in packed (3% EGSS-X) column. Silver resin chromatography (47 mm x 450 mm Michel-Miller column packed with 100% Ag^+/Na^+ resin; 5% acetonitrile (ACN) in methanol as solvent) and C18 reverse phase (RP) chromatography (50 mm x 250 mm stainless steel column, 5 micron particle size, Serva Feinbiochemica, Heidleberg, Germany; ACN as solvent) as described previously (12,13) was used for purification of the methyl arachidonate-d_4. The hydrogenation apparatus has been described elsewhere (14). ^{13}C-NMR spectra were obtained with a Bruker WH300 WB pulsed Fourier transform Spectrometer operating at 75.5 MHz.

Reagents. The following reagents were used as received: Dihydropyran, 2-propynol, Lindlar catalyst (5% palladium on calcium carbonate, poisoned with lead), triphenylphosphine, ethylmagnesium bromide (2.0 M in tetrahydrofuran (THF)), chromium trioxide (all Aldrich Chemical Co., Milwaukee, WI), lithium metal (Alfa Products, Danvers, MA), 2-pentynol, 5-hexynol (both Farchan Laboratories, Gainesville, FL), tris-triphenylphosphinechlororhodium (Strem Chemicals, Newburyport, MA), deuterium gas, 98% (Matheson Gas Products, Secacus, NJ) and hydrogen gas (Amerigas, Valley Forge, PA).

Methyl 5c,8c,11c,14c-eicosatetraenoate-17,17,18,18-d_4 (See Fig. 1).

2,5-Undecadiynol-8,8,9,9-d_4, 1. Propargyl alcohol (2-propynol; 22.9 g; 0.41 mol) in 250 mL THF was added to a 2 L, 3-necked round-bottomed flask equipped with a nitrogen inlet, thermometer, mechanical stirrer and syringe septum (all glassware heat-dried) and the solution was cooled to 8°C. Ethylmagnesium bromide (400 mL; 0.82 mol) was added via syringe over 1 hour at 10-20°C, and the thick slurry was stirred for 2 hr at room temperature. Cuprous chloride (1.0 g) was added, the slurry was stirred for 1 more hr and 1-bromo-2-octyn-5,5,6,6-d_4 (Prepared from 2-pentynol in a 5-step synthesis and in an overall yield of 61% as described in reference 11.) (52.5 g; 0.27 mol) in 40 mL of THF was added over 0.5 hr at room temperature. The slurry was stirred at reflux temperature for 21 hr (0.5 g more of cuprous chloride was added at 18 hr), then was cooled and the THF was removed by rotary evaporation. The residue was dissolved in 300 mL ether and 400 mL of 2N sulfuric acid was added with cooling. The layers were separated and the water layer was extracted with ether. The combined ether layers were washed with saturated ammonium chloride and water then dried over sodium sulfate. After filtration to remove the sodium sulfate, the solvents were removed by rotary evaporation and the residue was distilled through a short-path column to yield 22.6 g of 1 (95% pure; 50% yield; bp 98-102° C/0.05 mm Hg).

1-Bromoundeca-2,5-diyne-8,8,9,9-d_4, 2. Compound 1 (14.8 g; 0.09 mol) was reacted with triphenylphosphine and bromine as described previously for the 10-carbon analogue (15). Distillation of the residue (with 3.0 g silicon oil as "chaser") resulted in 17.4 g of 2 (90% pure; 82% yield; bp 91-100°C/0.10 mm Hg).

1-Iodoundeca-2,5-diyne-8,8,9,9-d_4, 3. Compound 2 (25.4 g; 0.10 mol) was dissolved in 250 mL acetone in a 500 mL, 1-necked round-bottomed flask. Sodium iodide (20.6 g; 0.14 mol) was added and the magnetically stirred solution was refluxed at 64°C for 1 hr. The solution was cooled, the precipitated sodium bromide was removed by vacuum filtration and the solvents by rotary evaporation. To the residue were added 200 mL each of ether and water, the layers were separated, and the water layer was extracted twice more

with ether. The ether layers were combined and washed with saturated sodium metabisulfite and water, and were dried over sodium sulfate. The drying agent was removed by vacuum filtration and the solvents by rotary evaporation to yield 30.2 g of 3 (86% pure; 95% yield).

5-Hexynoic acid, 4. Compound 4 (34.8 g; 95% pure; 57% yield; bp 70-78°C/0.2 mm Hg) was prepared by the chromium trioxide oxidation of 5-hexynol as described previously by Holland, et. al. for the 5-carbon analogue. (16).

5,8-Nonadiynoic acid, 5. Compound 5 (7.0 g; 98% pure; 44% yield; bp 112-114°C/0.10 mm Hg) was prepared by the Grignard coupling of compound 4 and 1-bromo-2-propyne as described in reference 17.

5,8,11,14-Eicosatetraynoic acid (ETYA), 6. Compound 5 (7.0 g; 0.046 mol) in 100 mL THF was added to a 300 mL, 3-necked round-bottomed flask equipped with a nitrogen inlet, thermometer, mechanical stirrer and septum inlet and cooled to 2°C by an ice/ salt bath. Ethylmagnesium bromide (47.3 mL; 0.092 mol; 2.0 M in THF) was added dropwise via syringe over 1 hr at 8-10°C and the slurry was stirred for 3 hr at room temperature. The slurry was cooled to 4°C, 150 mg cuprous cyanide was added and the stirring was continued for 15 min. The iodide, 3, (14.8 g; 0.046 mol) was then added dropwise over 30 min, the slurry was heated to 40°C for 1 hr and then stirred for 21 hr at room temperature (150 mg more cuprous cyanide was added after 18 hr). The slurry was poured into 150 mL of 2 N sulfuric acid (with cooling), the layers were separated and the water layer was extracted twice more with ether. The organic layers were combined and washed with 3 x 100 mL 2N ammonium hydroxide. The ammonium hydroxide washes were combined, acidified with 2N sulfuric acid and extracted three times with ether. The ether extracts were combined, washed with water and dried over sodium sulfate. The drying agent was removed by vacuum filtration and the solvents by rotary evaporator to yield 22.5 g of residue which was crystallized from 300 mL of 1:2 ether/PE V/V) twice to give 9.2 g of off-white, crystalline 6 [98% pure; 66% yield; mp 80-82°C (lit (17) 79.5-80.5°C)].

5c,8c,11c,14c-Eicosatetraenoic acid and methyl ester-17,17,18,18-d_4, 7 & 8. Benzene (120 mL), Lindlar Catalyst (0.6 g) and quinoline (1.8 mL) were combined in a 250 mL round-bottomed flask equipped with a magnetic stirrer. Compound 6 (1.5 g) was added and the semi-hydrogenation was started. Initial uptake of hydrogen gas was very slow. Two more batches of catalyst (0.3 & 0.1 g) were added to increase the rate of uptake. When uptake of hydrogen ceased, the catalyst was removed by vacuum filtration through a bed of Celite. The benzene was washed with 2% sulfuric acid and water, and was then dried over anhydrous sodium sulfate. The drying agent was removed by vacuum filtration and the solvents by rotary evaporation to yield crude 7 (1.53 g) which was esterified by addition of diazomethane in ether. The excess diazomethane was removed by flushing with nitrogen gas and the ether by rotary evaporator to yield 1.63 g of crude 8. Compound 8 was purified by C18 RP (ACN as solvent) and silver resin chromatography (5% ACN in methanol) to yield 1.2 g of 8 (98% pure; 75% yield).

RESULTS AND DISCUSSION

The synthetic sequence for methyl arachidonate-17,17,18,18-d_4 is given in Figure 1. The yields for each step are listed in brackets; the compound numbers are underlined. The overall yield was ca. 11%. The methyl arachidonate isotopic purity was determined to be 0.8% d_2, 6.7% d_3, 91.1% d_4, 1.2% d_5 and 0.2% d_7 with an average of 3.94 deuterium atoms per molecule. Table 1 lists the ^{13}C-NMR chemical shifts recorded for the tetraacetylenic precursor, 6, and methyl arachidonate, 8. The results correspond favorably for both the calculated shifts of the acetylenic carbons for compound 6 (18) and for compound 8 (19). Compound 6 had not been previuosly examined by ^{13}C-NMR. For both compounds, since the deuterium atoms were located on carbons 17 and 18, no chemical shifts were noted for these carbons (20).

In comparison to other methods of arachidonate syntheses (17,21-23), the synthetic scheme suggested by Dr. W-H Kunau (24) seems best. We found less oxidation and fewer difficulties in purification if no more than 2 or 3

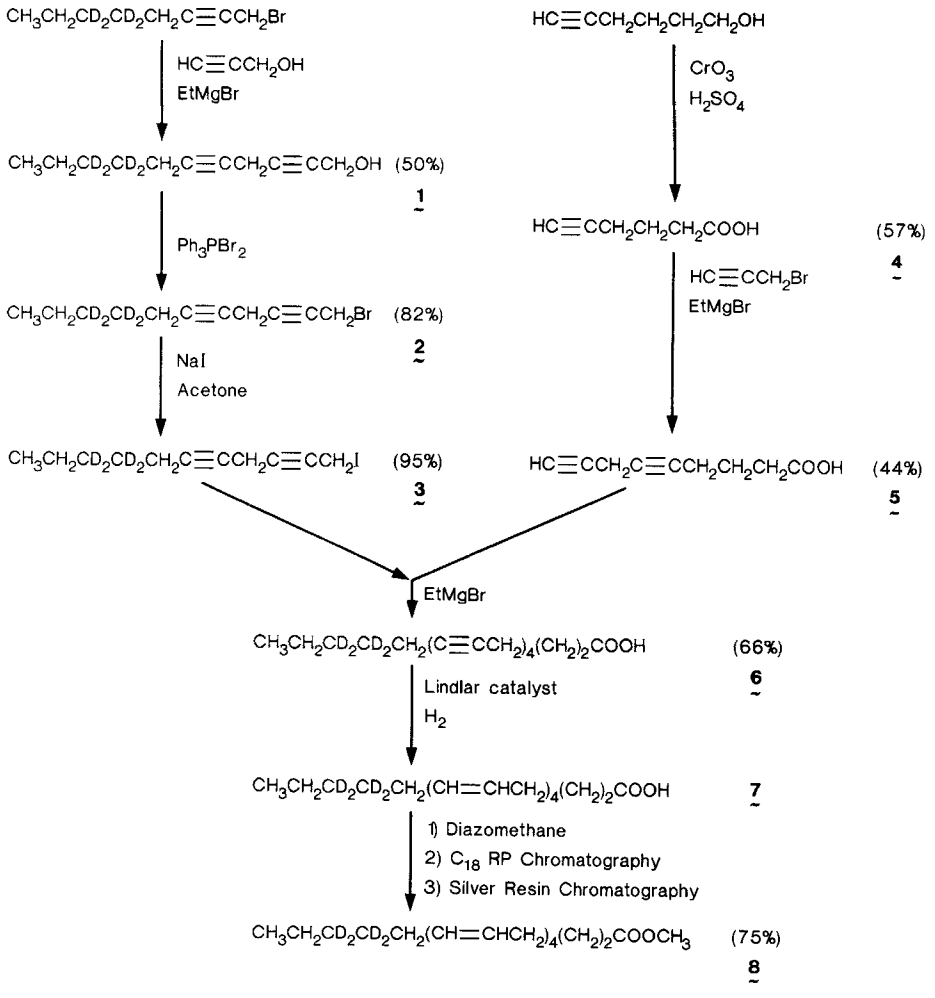

Figure 1: Synthetic scheme for methyl 5c,8c,11c,14c-eicosatetraenoate-17,17,18,18-d_4

acetylenic bonds were present in each fragment before the final Grignard coupling. Similar problems were encountered by van Dorp (22) and others who found "explosions" to sometimes occur during distillation as well as rapid polymerization of the triynoic alcohols (17). Silica gel chromatography was substituted for distillation where purity was not adversely affected; silicon oil was added as "chaser" when distillation was required. The iodide, 3, was used instead of the bromide, 2, in the final Wittig coupling reaction due to its greater reactivity (24).

The arachidonic acid-d_4 is best stored as ETYA, 6. This compound is very stable to both oxidation and polymerization and may be stored for months at -20°C with no apparent change in composition. Reduction to the tetra-olefinic acid, 7, and/or preparation of the methyl ester results in immediate loss of stability with an increase in color and oxidation side-products.

Table 1. ^{13}C-NMR chemical shifts for ETYA-17,17,18,18-d_4 and methyl arachidonate-17,17,18,18-d_4*

	ETYA		Methyl Arachidonate-d_4	
Carbon	Obs.	Calc (18)	Lit (19)	Obs
1	179.5	--	--	173.8
2	32.7	--	35.50	33.3
3	23.5	--	24.89	24.7
4	18.1	--	26.65	26.5
5	79.2	78.5	128.96	128.8
6	74.9	75.0	128.96	128.8
7	9.7	--	25.74	25.6
8	74.3	74.7	128.21	128.1
9	74.9	75.1	128.25	128.0
10	9.7	--	25.71	25.6
11	74.0	74.1	127.97	127.7
12	75.2	75.3	128.25	128.4
13	9.8	--	25.71	25.6
14	73.6	73.8	127.63	127.4
15	80.9	80.9	130.51	130.3
16	18.4	--	27.29	26.9
17	--	--	29.39	--
18	--	--	31.59	--
19	21.9	--	22.60	22.3
20	13.9	--	14.03	13.9
$-OCH_3$	--	---	--	51.3

* ppm downfield from $(CH_3)_4Si$

The Lindlar catalyzed semi-hydrogenation of ETYA, 6, proceeded slowly and required large amounts of catalyst. However, few isomers and little over-reduced material was noted. This contradicts previous findings (see ref. 24 & 25), but may be explained by better methods of catalyst preparation. The arachidonate was purified as the methyl ester because the ester was fractionated better than the acid by both C18 reverse phase and silver resin chromatography.

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