SYNTHESIS OF *cis* AND *trans* METHYL 8- AND 13-OCTADECENOATE-d_2 AND d_4 ISOMERS

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SUMMARY

The synthesis of deuterium labelled, unsaturated, isomeric fatty acids was required for a series of triple-labelled feeding experiments. The preparation of the *cis* and *trans* isomers of methyl 8-octadecenoate-17,18-d_2, methyl 8-octadecenoate-13,13,14,14-d_4, methyl 13-octadecenoate-17,18-d_2 and methyl 13-octadecenoate-17,17,18,18-d_4 is described. The syntheses utilize *tris*(triphenylphosphine)-chlororhodium(I) catalyst for the incorporation of deuterium. The double bond is introduced by coupling a deuterated alkyl triphenylphosphonium salt with the appropriate aldehydic ester using the Wittig reaction. Optimization of conditions has increased the overall yields for these syntheses to ~50% and the isotopic purity to >95%.

Key words: deuterium, fatty acids, methyl 8-octadecenoate, methyl 13-octadecenoate, Wittig reaction

INTRODUCTION

A study of the metabolism in man of *cis* and *trans* positional isomers of monoenoic fatty acids required the preparation of a series of di-, tetra-, and hexadeutero-octadecenoates. This paper describes the preparation and characterization of the di- and tetradeutero, *cis* and *trans*, methyl 8- and 13-octadecenoate isomers. These synthetic procedures are suitable for the preparation of d_2 and d_4 monoenoic fatty acids since they can be completed in approximately 3 weeks and use readily available chemicals. The deuterium atoms are incorporated several carbons away from the double bond in order to eliminate previously observed isotope effects due to deuterium on the double bond (1). A second problem of hydrogen-deuterium

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exchange when the deuterium is alpha to the double bond being formed in the final Wittig reaction is also eliminated by the synthesis described in this paper (2).

RESULTS AND DISCUSSION

The physical constants of the final products are given in Table I.

TABLE I: Physical Constants[a]

Compound[b]	Config.	Me Ester m.p. (°C)	Me Ester b.p. (mm Hg)	Acid m.p. (°C) Expt.	Acid m.p. (°C) Lit.[c]	Triglyceride m.p. (°C) Expt.	Triglyceride m.p. (°C) Lit.[d]
8-18:1-17,18-d_2	*cis*	-0.7	150-152 (0.01)	26.9	23.5-24	21.7	24
	trans	20.0		53.3	51.5-52.5	50.1	49
8-18:1-13,13,14,14,-d_4	*cis*	-2.4	151-153 (0.08)	27.4	23.5-24	24.6	24
	trans	19.3		52.8	51.5-52.5	52.3	49
13-18:1-17,18-d_2	*cis*	-8.9	144-146 (0.07)	30.5	26.5-27	27	26
	trans	10.4		45.8	43.5-44.5	45.9	44
13-18:1-17,17,18,18-d_4	*cis*	-11.0	154-155 (0.10)	27.8	26.5-27	26.3	26
	trans	10.1		42.1	43.5-44.5	43.9	44

[a] Melting points are ±0.5°C by differential thermal analysis.

[b] 8-18:1-17,18-d_2 = Methyl 8-octadecenoate-17,18-d_2.

[c] Melting points given for undeuterated compounds [see Barve, J. A., and F. D. Gunstone, Chem. Phys. Lipids 7:311 (1971)].

[d] Only the melting point of the β_1 crystalline form of the triglycerides is given. [see Hagemann, J. W., W. H. Tallent, J. A. Barve, I. A. Ismail, and F. D. Gunstone, J. Am. Oil Chem. Soc. 52:204 (1975)].

While the accuracy of the melting points (see Experimental section) is approximately ±0.5°C, larger variations were noted between the d_2 and d_4 isomers. Variations were more noticeable with the *cis* isomers and may be due to structural perturbations by the deuterium atoms.

Since these compounds were prepared using various reagents and workups, it is possible to compare the different synthetic steps for optimization of yields. The acetylenes can be reduced with Lindlar (3)

catalyst if the olefin is desired. The overall yields have been standardized depending on which starting material is used. If one starts with hydroxy-acetylenes or olefins, the overall yield is generally 50-60% (see preparation of *6*; Figure 1, Scheme A). Starting with chloroacetylenes or olefins and using the same Wittig coupling procedure as in *6* will result in an overall yield of 60-70% (see preparation of *12*; Figure 1, Scheme B).

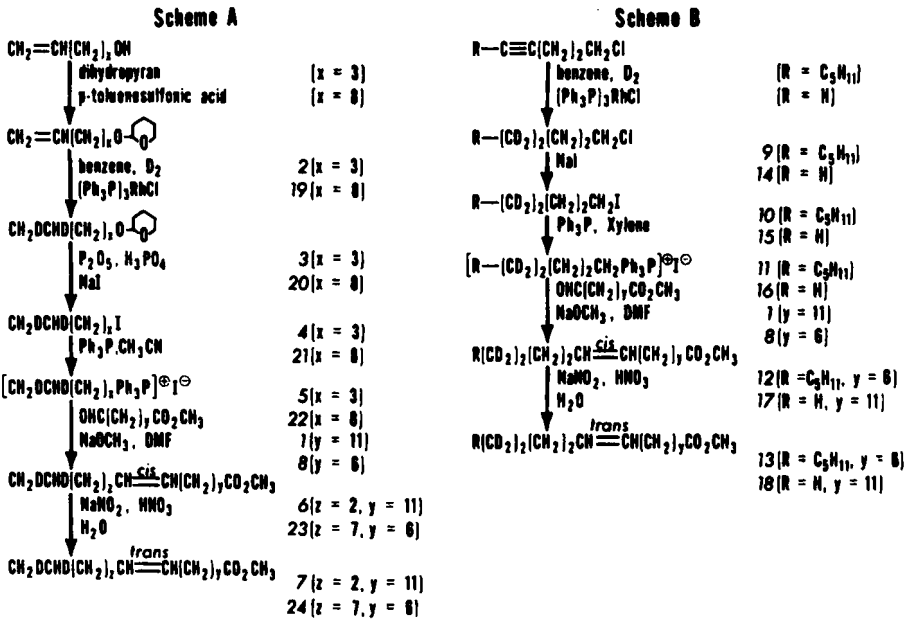

Figure 1. Synthetic scheme for Methyl 8- and 13-Octadecenoate-d_2 and d_4 Isomers

The deuterated tetrahydropyranyl ether or chloride intermediates can be analyzed by mass spectroscopy to determine the isotopic purity. These results (see Table II) agree within an error range of 1-5% with analyses of the final fatty acid esters. Some deuterium scattering is noticeable, but it can be attributed to hydrogen/deuterium exchange between the catalyst and the substrate during the deuteration step.

Variations of yield are most noticeable in the final Wittig coupling step of each synthesis, where the yields range from 42 to 75%. The yield increases from 42 to 56% if trimers are removed from the aldehydic

TABLE II: Mass Analyses for Deuterium

Compound	Number of Deuterium Atoms (%)							Average No. of Deuterium Atoms per Molecule
	0	1	2	3	4	5	6	
13-18:1-17,18-d_2[a]	2.3	1.8	93.2	0.5	2.1	0.1	0.0	1.99
2-(Pentyloxy-4,5-d_2)-tetrahydropyran	0.2	1.7	94.0	3.8	0.0	0.3	0.0	2.03
13-18:1-17,17,18,18-d_4	5.2	1.7	3.2	1.9	87.5	0.3	0.3	3.65
1-Chloropentane-4,4,5,5-d_4	2.6	0.0	5.4	0.0	88.9	3.0	0.0	3.81
8-18:1-13,13,14,14-d_4	0.7	0.3	1.4	3.2	93.0	1.1	0.3	3.92
1-Chlorodecane-4,4,5,5-d_4	1.2	2.6	4.2	1.3	90.5	0.2	0.0	3.78
8-18:1-17,18-d_2	3.8	1.7	92.1	1.6	0.4	0.3	0.0	1.94
2-(Decyloxy-8,9-d_2)-tetrahydropyran	1.3	0.9	97.5	0.0	0.1	0.3	0.0	1.98

[a] 13-18:1-17,18-d_2 = methyl 13-octadecenoate-17,18-d_2.

ester by distillation or hydrolysis prior to the final Wittig coupling (4). Yields can further be increased to 70 or 75% if dry, commercial $NaOCH_3$ is used.

These synthetic procedures are valuable for the preparation of d_2 and d_4 monoenoic fatty acids. Double bond position may be varied by simply changing the number of carbons in the aldehydic ester and alkyl triphenylphosphonium iodide fragments to be used in the final Wittig coupling.

EXPERIMENTAL

Instruments--A Nuclide 12-90G spectrometer with 70 eV impact ionization inlet, maintained at a temperature of 150°C for the intermediates and 200°C for the methyl octadecenoates, was used to determine isotopic purity and deuterium distribution (5). *cis* and *trans* percentages were determined with a Packard Model 7400 Gas Chromatograph equipped with a

20 ft X 4 mm glass column packed with 15% OV-275. Flame ionization detectors (FID) and He carrier gas were used (6). Purities of intermediates were determined on an Aerograph 600-B Gas Chromatograph equipped with FID and N_2 carrier gas. A 12 ft X 1/8 in. stainless-steel column packed with 3% EGSS-X was used.

The methyl 8-octadecenoate-d_2 intermediates were analyzed on an HP 5700 Gas Chromatograph equipped with a 6 ft X 1/8 in. stainless-steel column packed with 10% OV-101. FID and He carrier gas were employed. The melting points of the octadecenoates were determined on a DuPont 900 Differential Thermal Analyzer using silicon carbide as reference.

Reagents--The following reagents were used as received: 9-Decen-1-ol (Aldrich), 1-Chloro-4-decyne (Farchan Chemical Company), 1-Chloro-4-pentyne (Farchan Chemical Company), 4-Penten-1-ol (PCR, Inc.), Triphenylphosphine (Aldrich), Silica Gel (60-200 mesh) (Baker), Sodium Methoxide (Harshaw Chemical Company), *Tris*(triphenylphosphine)-chlororhodium(I) (Strem Chemicals), and Deuterium (98.0%) (Matheson). Other chemicals used were "ACS Certified" and, unless specifically stated, were used without further purification.

Methyl 12-Formyldodecanoate, *1*: Compound *1* was prepared by the ozonolysis of methyl erucate in methanol as previously described (7) (b.p. of methyl ester 138-142°C/0.007 mm Hg).

2-(4-Pentenyloxy)tetrahydropyran, *2*: The tetrahydropyranyl (THP) ether of 4-pentene-1-ol was prepared by mixing 4-pentene-1-ol (51 g; 0.59 mol), dihydropyran (84.7 g; 1.01 mol), and p-toluenesulfonic acid (5.0 g) in 500 ml of diethyl ether (Et_2O) (8). The mixture was stirred in a water bath at room temperature for 20 hr, then analyzed for *2* by gas chromatography (GC) (90°C). Saturated sodium bicarbonate solution (200 ml) and solid sodium carbonate (Na_2CO_3; 5.0 g) were then added. The solution was transferred to a separatory funnel with Et_2O, then washed four times with 300-ml portions of water. The Et_2O layer was dried over sodium sulfate (Na_2SO_4) and filtered, and the solvent was removed by a rotary evaporator. Distillation of the residue through a

6-in. Vigreux column (64.5-67.0°C/0.85 mm Hg) gave 97.7 g of *2* (98% pure), for a yield of 96%.

2-(Pentyloxy-4,5-d_2)tetrahydropyran, *3*: Compound *2* (87.0 g; 0.50 mol) was added to 750 ml of degassed (argon) and predried ($CaSO_4$) benzene in a 2-liter round-bottomed flask equipped with a magnetic stirrer and gas inlet (9,10). The system was flushed with deuterium, 8.0 g of $(Ph_3P)_3RhCl$ (Wilkinson's Catalyst) was added, and the system was flushed again with deuterium. While a positive pressure of deuterium was maintained (775 mm Hg), the solution was stirred vigorously until deuterium uptake ceased (~2 hr). An ice bath was needed at the onset of D_2 uptake to maintain the temperature at ~25°C. A rotary evaporator was used to remove ~400 ml of the benzene. The remainder of the solution was diluted with 1.5 liter of petroleum ether (PE) and eluted through a 5.5 X 50 cm column containing 225 g of silica gel A. Evaporation of the solvent resulted in 83.4 g of *3* (99% pure; 96% yield).

1-Iodopentane-4,5-d_2, *4*: A 1-liter, three-necked round-bottomed flask containing phosphorous pentoxide (84.5 g; 0.60 mol) was equipped with an N_2 inlet, thermometer, addition funnel, and mechanical stirrer (11). Phosphoric acid (85% H_3PO_4; 295.0 g; 2.56 mol) was slowly added during which the temperature rose to 145°C. After cooling by ice bath to ~45°C, sodium iodide (412.0 g; 2.75 mol) was added and the mixture was stirred for 1/2 hr. Compound *3* (78.0 g; 0.49 mol) was added dropwise over a 45-min period. The reaction mixture was stirred at 80-90°C for 4 hr and at room temperature overnight. The reaction mixture was transferred to a separatory funnel, and 0.5 liter of PE and 1.0 liter Et_2O were added. Solid sodium thiosulfate (~1 g) was added and the solution was washed three times with 250-ml portions of saturated sodium chloride solution. The PE/Et_2O layer was dried over Na_2SO_4, then filtered, and the solvent was removed by a rotary evaporator. The residue was distilled through a short path column (23-24°C/0.47 mm Hg) to give 71.3 g of compound *4*. The deep red iodide color was removed by redissolving *4* in ether, washing with sodium thiosulfate, drying over Na_2SO_4, filtering,

and evaporating the solvent. This resulted in 70.0 g of compound *4* (98% pure). Redistillation of the solvent removed earlier by a rotary evaporator resulted in recovery of an additional 12 g of compound *4* (>98% pure). Overall total yield of *4* was 90%.

1-Pentyl-4,5-d_2-triphenylphosphonium iodide, *5*: Compound *4* (70.0 g; 0.35 mol), triphenylphosphine (102.0 g; 0.39 mol), and acetonitrile (CH_3CN; 550 ml) were combined in a 1-liter round-bottomed flask equipped with a reflux condenser and N_2 ebullator. The magnetically stirred solution was refluxed overnight, then cooled, and the CH_3CN was removed by a rotary evaporator. The solid residue was transferred to a 1-liter beaker and washed twice with 500-ml portions of Et_2O. The mixture was filtered and the solid washed with Et_2O and dried in a vacuum desiccator. This gave 148.9 g (94% yield) of compound *5* (m.p. 169-170°C).

Methyl 13-Octadecenoate-17,18-d_2, *6*: Compound *5* (148.9 g; 0.32 mol) in 325 ml N,N-dimethylformamide (DMF) was added dropwise over 1/2 hr to a 1-liter, three-necked, round-bottomed flask that contained sodium methoxide ($NaOCH_3$; 17.4 g; 0.32 mol) and that was equipped with thermometer, N_2 inlet, addition funnel, and mechanical stirrer. The bright orange mixture was stirred for 30 min, then cooled to 9°C by use of an ice bath. Methyl 12-formyldodecanoate (Compound *1*; 81.7 g; 0.32 mol) in 200 ml DMF was then added dropwise over 1/2 hr, while the temperature was maintained below 18°C. The ice bath was removed, and the solution was stirred for 1.5 hr and then transferred to 1.6 liter of 0.1 N HCl at 10°C.

A white precipitate formed which was removed by vacuum filtration. The H_2O layer was extracted three times with 350-ml portions of PE; the PE layers were combined and dried over Na_2SO_4. Filtration and solvent evaporation yielded 104.6 g of product which was distilled through a 3-in. Vigreux column. The fractions were collected and gave 75.2 g of compound *6* (95+% pure, 75% yield). GLC analysis showed 93% *cis*, 7% *trans*.

Methyl 13-*trans*-Octadecenoate-17,18-d_2, *7*: The *trans* isomer was prepared by the isomerization of compound *6* (12). Compound *6* (60.0 g; 0.20 mol) and nitric acid (4.13 g) in 4.70 ml H_2O (0.046 moles HNO_3 as 6 M solution) were added to a 500-ml, three-necked round-bottomed flask equipped with a mechanical stirrer, N_2 inlet, thermometer, and addition funnel. The mixture was heated to 67°C and sodium nitrite ($NaNO_2$; 1.59 g; 0.023 mol) in 9.80 ml H_2O was added dropwise over a period of 5 min. After about 2 hr of stirring at 68-70°C, the solution was transferred with 600 ml PE to a separatory funnel and washed three times with 160-ml portions of H_2O. The PE layer was then dried over Na_2SO_4. The Na_2SO_4 was removed by vacuum filtration and the PE was evaporated by a rotary evaporator. The residue was eluted by benzene through a 30 X 570 mm column containing 100 g of silica gel. Removal of the solvent by a rotary evaporator gave 55.4 g (92% yield) of product (79% *trans*/21% *cis*). The *cis* and *trans* isomers (20-g batches) were separated by methanol elution on a 300 X 5 cm silver resin column (13). The purity of the *trans* isomer was >99%.

Methyl 7-Formylheptanoate, *1*: The acid was prepared by the ozonolysis of cyclooctene according to the method of Siclari and Rossi (14). Esterification using methanol/trimethylorthoformate/HCl produced the corresponding acetal ester which was hydrolyzed with water/acetonitrile/HCl just before use (4) (b.p. ester, 130-134°C/0.04 mm Hg).

1-Chlorodecane-4,4,5,5-d_4, *9*: 1-Chloro-4-decyne (172 g; 1.0 mol) was deuterated in 1100 ml benzene using 8.0 g of Wilkinson's catalyst (see synthesis of compound *3*). The final solvent removal gave 179 g of *9* (99% yield).

1-Iododecane-4,4,5,5-d_4, *10*: Compound *9* (176.0 g; 0.98 mol), NaI (225 g; 1.5 mol) and 1100 ml of methyl ethyl ketone (MEK) were combined and refluxed overnight (15). The precipitated sodium chloride was removed by filtration and the solvent was evaporated under vacuum. The residue was added to 1100 ml H_2O and extracted three times with 300-ml portions of Et_2O. The Et_2O fractions were combined, washed with 150 ml

saturated sodium thiosulfate and 2 X 100 ml H_2O, and dried over Na_2SO_4. The Na_2SO_4 was removed by vacuum filtration and the Et_2O by rotary evaporation to give 253.4 g of *10* (99% pure; 93% yield).

1-Decyl-4,4,5,5-d_4-triphenylphosphonium iodide, *11*: Compound *10* (140.1 g; 0.52 mol) and triphenylphosphine (148.4 g; 0.57 mol) were dissolved in 1000 ml xylene and refluxed under N_2 overnight as in the preparation of *5*. A similar workup gave 249.9 g of *11* (90% yield).

Methyl 8-Octadecenoate-12,12,13,13-d_4, *12*: Compound *11* (369.4 g; 0.69 mol) was dissolved in 1600 ml DMF and $NaOCH_3$ (54 g; 1.0 mol) was added. The $NaOCH_3$ was prepared by dissolving 23 g of sodium metal in 1000 ml methanol and subsequently removing solvent by rotary evaporator. The mixture of the phosphonium iodide and $NaOCH_3$ was stirred for 1-1/2 hr over molecular sieve 3A, and *8* (200.8 g; 0.77 mol) was slowly added over 1-1/4 hr. The mixture was maintained at room temperature during the addition by an ice bath and stirred overnight. The mixture was added to 2000 ml of 0.1 N HCl and extracted three times with 300-ml portions of PE and once with 300 ml Et_2O. The extractions were combined, dried over Na_2SO_4, filtered, and the solvent was removed by a rotary evaporator. Distillation of the residue through a 6-in. Vigreux column gave 93.7 g of *12* (95% pure; 42% yield). GC analysis indicated 93% *cis*/7% *trans* isomers.

Methyl *trans*-8-Octadecenoate-12,12,13,13-d_4, *13*: Isomerization was accomplished as for *7*. The yield was 89% of a mixture containing 82% *trans* and 18% *cis* of the isomer. Separation of the isomers was made by silver resin chromatography. The purity of the *trans* isomer was >99%.

1-Chloropentane-4,4,5,5-d_4, *14*: See preparation of *9*. Due to the high volatility of *14*, the volume of the solvent plus sample was decreased by a rotary evaporator to ∿250 ml (∿20-30% product loss by GC analysis of the distillate). The sample was used without further isolation in the next preparation.

1-Iodopentane-4,4,5,5-d_4, *15*: The iodide was prepared from *14* in a manner similar to *10*, except that acetone was used as the solvent and

the sample was extracted with hexane. High volatility again caused sample losses (10-15%) during removal of the hexane, so the volume could only be reduced to ∿550 ml.

1-Pentyl-4,4,5,5-d_4-triphenylphosphonium iodide, *16*: The above compound was prepared as previously described for *11*. Isolation of the reaction product gave 0.48 mol of *16* (m.p. 145-165°C).

Methyl 13-Octadecenoate-17,17,18,18-d_4, *17*: The procedure was identical to that used for the preparation of *6* except that the methyl-12-formyl dodecanoate (1) was distilled just before use. Triphenylphosphonium iodide *16* (223.5 g; 0.48 mol), $NaOCH_3$ (40 g; 0.65 mol), compound *1* (133 g; 0.58 mol), and 1200 ml of DMF were used. The boiling point of *17* was 154-155°C/0.1 mm Hg and the yield was 56% (91% *cis*/9% *trans*).

Methyl *trans* 13-Octadecenoate-17,17,18,18-d_4, *18*: See preparation of *7*. The isomerization of 40.0 g of *17* gave 36.8 g of *18* (80% *trans*/20% *cis*) and a yield of 92%. Separation of the isomers was made by silver resin chromatography. The purity of the *trans* isomer was >99%.

2-(9-Decenyloxy)tetrahydropyran, *19*: Compound *19* was prepared as described for *2*. The yield was 99% and the boiling point 108-110°C/0.05 mm Hg.

2-(Decyloxy-9,10-d_2)tetrahydropyran, *20*: Compound *20* was prepared as described for *3*. Compound *19* (92 g; 96.2% pure; 0.37 mol) was deuterated over 8.0 g Wilkinson's catalyst in 800 ml benzene for 4 hr. Workup gave 84 g of *20* (98.8% by GLC) and a yield of 92%.

1-Iododecane-9,10-d_2, *21*: Compound *20* (83 g; 0.3 mol) was combined with H_3PO_4 (223 g; 1.9 mol), P_2O_5 (68 g; 0.5 mol), NaI (313 g; 2.1 mol), and heated to 130°C. Workup and distillation as described for *4* (88°C/0.07 mm Hg) gave 79 g (98.5% pure) of *21* and a yield of 85%.

1-Decyl-9,10-d_2-triphenylphosphonium iodide, *22*: Compound *21* (78 g; 0.28 mol) and Ph_3P (7.9 g; 0.3 mol) were combined in 500 ml CH_3CN as described for the preparation of *5*. Workup gave 147 g of *22* (m.p. 83-85°C) and a yield of 97%.

Methyl 8-Octadecenoate-17,18-d_2, *23*: The preparation of *23* is similar to *6*. However, the molar ratios, temperatures, and pH of the water to which the mixture is added are different. The phosphonium iodide *22* (147 g; 0.28 mol), $NaOCH_3$ (14 g; 0.26 mol) and *8* (53 g; 82.7% pure; 0.25 mol) were combined with 450 ml DMF while the temperature was maintained at 18-28°C. The reaction mixture was added to 1000 ml H_2O and workup was as described for *6*. Distillation of *23* gave 43 g (97.2% pure) of *22* and a yield of 56% (90% *cis*/9% *trans*).

Methyl *trans*-8-Octadecenoate-17,18-d_2, *24*: The *cis* isomer *23* (25.3 g) was isomerized as described for *7*. Product analysis by GC gave 24.6 g of *24* (32% *cis*/68% *trans*) and a yield of 94%. Separation of the isomers was made by silver resin chromatography. The purity of the *trans* isomers was >99%.

Preparation of Fatty Acids and Triglycerides: Compounds *6*, *7*, *12*, *13*, *17*, *18*, *23*, and *24* were saponified using alcoholic KOH (95-99% yield). The triglycerides of these compounds were prepared by the p-toluenesulfonic acid catalyzed esterification of the fatty acids with glycerol (~90% yield) as described previously (16).

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