Preparation of Deuterium-Labelled Methyl Linoleate and Its Geometric Isomers from Natural Seed Oils[1]

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Multi-gram quantities of deuterium-labelled methyl linoleate (methyl *cis*-9,*cis*-12-octadecadienoate) and its geometric isomers are readily synthesized from *Crepis alpina* (70–80% *cis*-9-octadecen-12-ynoic acid) and *Vernonia galamensis* (70–80% 12,13-epoxy-*cis*-9-octadecenoic acid) seed oils. Methyl *cis*-9,*cis*-12- and *trans*-9,*cis*-12-octadecadienoate-12,13-d_2 were prepared by the Lindlar-catalyzed reduction (with D_2 gas) of methyl *cis*-9- and *trans*-9-octadecen-12-ynoates, respectively. Methyl *trans*-9-octadecen-12-ynoate was synthesized by the *p*-toluenesulfinic acid-catalyzed isomerization of the corresponding *cis* isomer. Methyl *cis*-9,*cis*-12-; *trans*-9,*cis*-12; *cis*-9,*trans*-12- and *trans*-9, *trans*-12-octadecadienoate-d_2, d_4 and d_6 were prepared by the Wittig coupling (with stereochemical control) of the appropriate d_2-, d_4- or d_6-alkyltriphenylphosphonium salt with methyl 12-oxo-*cis*-9- or *trans*-9-dodecenoate (prepared by the para-periodic acid cleavage of methyl 12,13-dihydroxy-*cis*-9- or *trans*-9-octadecenoate). The *cis* dihydroxy ester was synthesized from *Vernonia galamensis* seed oil by acetolysis, saponification and then esterification. The *cis* dihydroxy ester was isomerized by nitric acid/sodium nitrite to the *trans* form and purified by silver resin chromatography. Isotopic purities ranged from 88% (for the d_6 isomers) to 99% (for the d_2 isomers).

KEY WORDS: *Cis* and *trans* isomers, *Crepis* oilseeds, deuterium, fatty esters, linoleic acid and esters, preparation, vernolic acid and *Vernonia* oil, Wittig.

Substantial quantities of deuterium-labelled methyl linoleate [methyl *cis*-9,*cis*-12-octadecadienoate (9c, 12c-18:2)] and the *trans* linoleate isomers are required to investigate the metabolism of these fatty acids in humans (1,2) and animals (E.C. Beyers, and E.A. Emken, submitted for publication). Several natural seed oils were utilized as precursors for the rapid preparation of both labelled and unlabelled linoleate isomers. The most useful seed oil sources were *Crepis alpina* [14–16% oil; 70–80% crepenynic acid (*cis*-9-octadecen-12-ynoic acid)] and *Vernonia galamensis* [40–42% oil; 70–80% vernolic acid (12,13-epoxy-*cis*-9-octadecenoic acid)]. The crepenynic acid was isolated from *Crepis alpina* and used for the rapid and high yield preparation of multi-gram quantities of methyl *cis*-9,*cis*-12-octadecadienoate-12,13-d_2 and methyl *trans*-9,*cis*-12-octadecadienoate-12,13-d_2. These deuterium-labelled fats were synthesized more rapidly and in higher yield than by currently available methods (3–5). Vernolic acid isolated from *Vernonia galamensis* was used as a convenient precursor for the key intermediate, 12,13-dihydroxy-9-octadecenoate. The aldehydic ester prepared from this intermediate was used for the synthesis of a variety of di-, tetra- and hexadeuterated methyl 9,12-octadecadienoate *cis* and *trans* isomers.

EXPERIMENTAL PROCEDURES

Materials. 3-Hexynol (Farchan Laboratories, Gainesville, FL); Lindlar Catalyst, n-butyl lithium (2.6M in hexane), *p*-toluenesulfinic acid (all Aldrich Chemical Co., Milwaukee, WI); *tris*(triphenylphosphine)-rhodium(I) chloride (Strem Chemicals, Newburyport, MA) and deuterium gas, 98% (Matheson Gas Products, Secaucus, NJ) were purchased from commercial sources.

Methods. Methyl *cis*-9-octadecen-12-ynoate was prepared by the transesterification (sodium metal/methanol) of *Crepis alpina* seed oil with purification by silver resin chromatography (6). As described previously, methyl *threo*-12,13-dihydroxy-*cis*-9-octadecenoate was prepared by acetolysis, saponification and then esterification of *Vernonia galamensis* (or *anthelmintica*) seed oils (7,8). The *cis* or *trans* methyl 12-oxo-9-dodecenoates were generated by the para-periodic acid cleavage of *cis* or *trans* methyl *threo*-12,13-dihydroxy-9-octadecenoates (9). The methyl *threo*-12,13-dihydroxy-*trans*-9-octadecenoate was prepared by the nitrous acid-catalyzed isomerization of the corresponding *cis* isomer (10). *p*-Toluenesulfinic acid was prepared by the method of Kice *et al.* (11). The triphenylphosphonium salts of 1-iodohexane-3,3,4,4-d_4 (12) and 1-iodohexane-5,5,6,6-d_4 (13) were synthesized as described previously.

Instrumentation. Gas liquid chromatographic (GLC) analyses were conducted on a Varian 3400 gas chromatograph (GC) (He carrier gas; flame ionization detection) equipped with a .25 mm × 30 m SP2330 capillary column (Supelco Inc., Bellefonte, PA). Isotopic purity and deuterium distribution were determined on a Finnigan 4500 mass spectrometer (MS) interfaced with a GC equipped with a Supelcowax 10 capillary column (Supelco) (0.32 mm × 0.5 micron film thickness) and programmed from 165°C to 265°C at 5°C/min. Helium was used as carrier gas and samples were analyzed by chemical ionization (conditions: isobutane reagent gas, 70 ev). The preparation and utilization of silver resin chromatography has been described elsewhere (6).

Synthesis of methyl cis-9,cis-*12- and trans-9,cis-12-octadecadienoates-12,13-d_2 (Fig. 1):*

Methyl cis-9,cis-*12-octadecadienoate-12,13-d_2, 1.* Lindlar catalyst (0.43 g) and 125 mL hexane were combined in a 250-mL round-bottomed flask (oven-dried) and the system was degassed 3 times with deuterium (D_2)/house vacuum. Freshly distilled quinoline (0.7 mL) was added and the system was degassed twice more. After 10 min of stirring, methyl *cis*-9-octadecen-12-ynoate (8.42 g; 99% pure; 29 mmol) was added and the system degassed with D_2 three more times. With vigorous stirring, D_2 uptake was complete within 1 hr. The reaction mixture was filtered through a bed of Celite, transferred to a separatory

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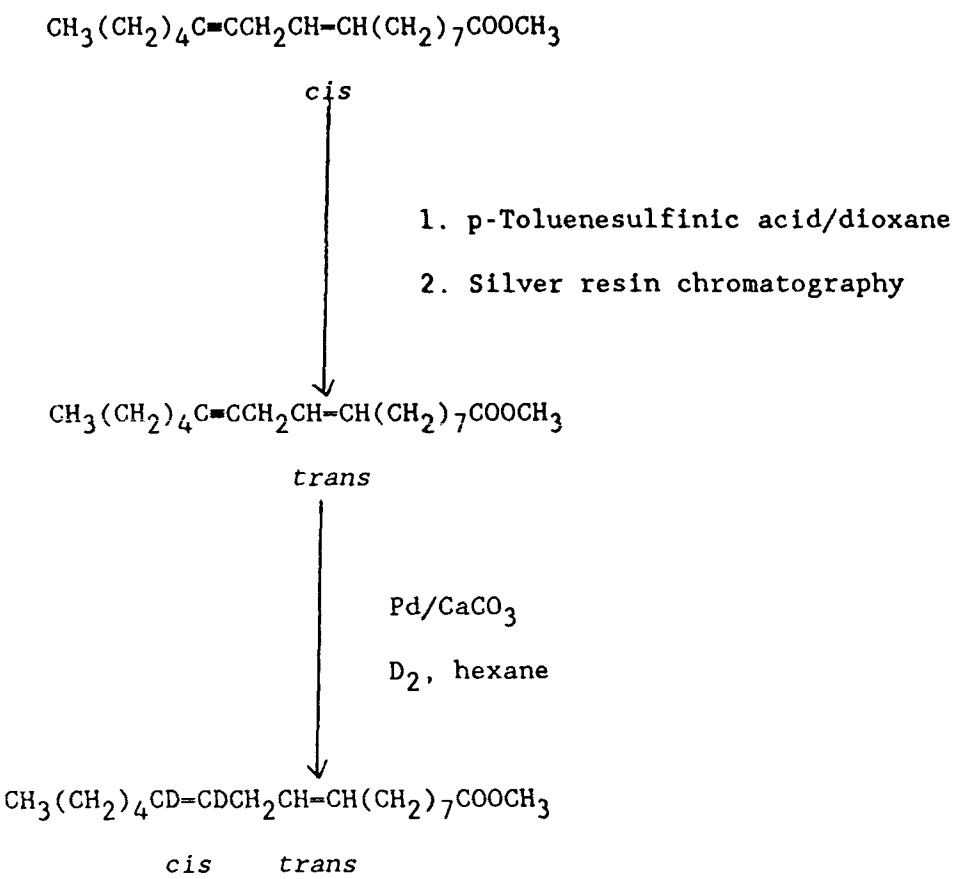

FIG. 1. The synthesis of methyl *trans*-9,*cis*-12-octadecadienoate-12,13-d_2.

funnel, and washed with 5% hydrochloric acid and then water. The solution was dried over sodium sulfate, vacuum filtered and the solvent was removed by rotary evaporation to yield 8.2 g of **1** (99% pure by GC, 98% yield).

Methyl trans-*9*,cis-*12-octadecadienoate-12,13-d_2,2.* Methyl *cis*-9-octadecen-12-ynoate (9.7 g), dioxane (400 mL) and *p*-toluenesulfinic acid (TSA; 1.0 g) were combined in a 500-mL round-bottomed flask equipped with a reflux condenser and N_2 inlet (14,15). The solution was stirred magnetically and heated by an oil bath to reflux at 110°C. GC analysis was used to follow the *cis/trans* conversion. After 1 hr, only 10% *trans* was found. At 1-hr intervals, 3 more batches of TSA were added (900, 900 and 500 mg). GC analysis indicated an increase of *trans* to 53%, 71% and finally 74% (4 1/2 hr total reaction time). The solution was cooled and transferred to a separatory funnel with petroleum ether (PE) and 500 mL 1N sodium hydroxide. The isomer mixture was extracted three times with PE, the PE layers were combined and washed three times with water and then dried over sodium sulfate. The sodium sulfate was removed by vacuum filtration and the solvents by rotary evaporation. The residue was chromatographed through a 4 × 60 cm glass column packed with 100% silver resin (7 mL methanol/min) to yield 6.6 g of the *trans* isomer (99% pure; 67% yield). The combined *cis* and *trans* recovery was >91%. The *trans* isomer (6.0 g) was then reduced with Lindlar catalyst (see preparation of compound 1) to yield 6.0 g compound **2** (98% chemically pure; 98% yield).

Preparation of the di-, tetra- and hexadeuterated triphenylphosphonium halides utilized in the synthesis of deuterium-labelled linoleate isomers (Fig. 2):

3,4-Dideutero-(compound 3), 3,3,4,4-tetradeutero-(compound 4) and 4,4,5,5-tetradeuterotriphenylphosphonium iodide (compound 5). Compounds **3**, **4** and **5** were prepared from 3-hexenol, 3-hexynol and 5-hexynol, respectively, in overall yields of 65–69% as described previously (12).

2,2,3,3,5,6-Hexadeuterohexyltriphenylphosphonium iodide, 6. Compound **6** was prepared as previously described for the 9-carbon analogue (16). 3-Bromopropene was coupled via the Grignard Reaction with 2-(2′-propynyloxy)-tetrahydropyran to produce 2(5′-hexen-2′-ynyloxy)-tetrahydropyran (80% yield; b.p. 75–78°C/0.05 mmHg). Reduction with D_2 gas and *tris*-triphenylphosphine-rodium(I) chloride yielded 2-(1′-hexyloxy-2,2,3,3,5,6-d_6) tetra-hydropyran (93% yield; b.p. 69–74°C/0.8 mmHg). The hexadeuterated compound was converted to 1-iodohexane-2,2,3,3,5,6-d_6 (87% yield; b.p. 65–66°C/12 mmHg) by phosphoric acid/sodium iodide and then heated at reflux with triphenylphosphine in acetonitrile to produce compound **6** (96% yield; m.p. 142–144°C).

The synthetic scheme for preparation of the di-, tetra- and hexadeuterated octadecadienoates is presented in Figure 2. The appropriate di-, tetra- and hexadeuterated 6-carbon triphenylphosphonium halide was coupled with 12-oxo-*cis*-9 or *trans*-9-dodecenoate via the Wittig reaction. The Wittig reaction was stereochemically controlled to yield predominantly the *cis* (17) or *trans* (17,18)

$CX_3(CX_2)_5PPh_3^+I^-$ $OHCCH_2CH{=}CH(CH_2)_7COOCH_3$

i-3,4-d_2(3) I-*cis*

ii-3,3,4,4-d_4(4) II-*trans*

iii-5,5,6,6-d_4(5)

iiii-2,2,3,3,5,6-d_6(6)

(X= H or D) A = n-BuLi/ THF/ 6°C

B = n-BuLi/ diethyl ether

-40°C, then CH_3OH

$CH_3(CH_2)_4HC{=}CHCH_2CH{=}CH(CH_2)_7COOCH_3$

Phosphonium Salt	Aldehydic Ester	Reaction Conditions	Methyl Ester % Product	Co-product
i	I	A	99% 9c,12c-18:2-15,16-d_2	-
i	II	A	99% 9t,12c-18:2-15,16-d_2	-
i	II	B	50% 9t,12t-18:2-15,16-d_2	9t,12c
ii	I	B	60% 9c,12t-18:2-15,15,16,16-d_4	9c,12c
iii	I	A	92% 9c,12c-18:2-17,17,18,18-d_4	9c,12t
iiii	I	A	93% 9c,12c-18:2-14,14,15,15, 17,18-d_6	9c,12t

FIG. 2. Preparation of linoleate and linoleate isomers by the Wittig coupling of the appropriate aldehydic ester and deuterium-labelled phosphonium salts.

double bond at the 12-position. Final purification was by silver resin chromatography. Overall yields varied from 17 to 39% of the desired isomer.

RESULTS AND DISCUSSION

The synthesis of *trans*-9,*cis*-12-octadecadienoate-12,13-d_2 from methyl *cis*-9-octadecen-12-ynoate is presented in Figure 1. Without the initial isomerization step, the same procedure was used to prepare methyl *cis*-9, *cis*-12-octadecadienoate-12,13-d_2. The *p*-toluenesulfinic acid-catalyzed isomerization of methyl *cis*-9-octadecen-12-ynoate required several batches of catalyst and some 4 1/2 hr to achieve a 74% *trans*/26% *cis* ratio. One batch of the same catalyst quickly isomerized methyl *cis*-9-octadecenoate within one hour. With methyl *cis*-9-octadecen-12-ynoate, heating at reflux a similar catalyst/substrate ratio overnight only increased the *trans*/*cis* ratio from 10/90 to 17/83. The same was true

TABLE 1

Mass Analysis for Deuterium

Linoleate configuration		No.	Deuterium	Number of deuterium atoms (%)							
9	12	deuterium	positions	0	1	2	3	4	5	6	7
cis	*cis*	2	12,13	—	0.6	99.1	0.2	—	—	—	—
trans	*cis*	2	12,13	2.9	—	95.9	1.3	—	—	—	—
trans	*trans*	2	12,13	0.1	2.9	97.0	—	—	—	—	—
cis	*trans*	4	15,15,16,16	0.3	0.3	—	2.7	96.2	0.5	—	—
cis	*cis*	4	17,17,18,18	—	—	1.4	1.4	96.7	0.2	0.2	—
cis	*cis*	6	14,14,15,15,17,18	—	0.1	—	1.4	0.2	7.1	88.8	2.4

when the amount of catalyst was increased four-fold. The same results were obtained, even after multiple purifications of the starting material. Multiple additions of catalyst were required, but overall yields were not affected. Silver resin chromatography was used to isolate the *trans* from the *cis* isomer. *p*-Tbluenesulfinic acid was utilized instead of sodium nitrite/nitric acid for isomerization because the former provided consistently higher yields and did not discolor the substrate. The Lindlar-catalyzed reduction of the *cis*-9 or *trans*-9-octadecen-12-ynoate proceeded with little over- or under-reduction (ca. 1–2%). In most instances, purification by silver resin chromatography of the reduced product was not necessary. As noted in Table 1, the isotopic purities of the dideuterated linoleate isomers were consistently high (96–99 + % d_2). We have found no isotope effects in biological studies due to the deuterium atoms located on the 12- and 13-carbons.

A wide variety of deuterium-labelled, 6-carbon triphenylphosphonium salts may be prepared from the appropriate 6-carbon unsaturated alcohol or chloride (bromides and iodides cannot be reduced with the rhodium catalyst). These compounds are widely available. The location of the deuterium atoms can be varied from the Δ2 to the Δ5 position (C-14 to C-18 of the 9,12-octadecadienoates) by careful choice of the starting material. Only the hexadeutero precursor was not readily available and had to be synthesized. Overall yields of the rhodium-catalyzed reduction of the unsaturated tetrahydropyranyl ethers, conversion to the iodides and phosphonium salt preparations were similar to those yields described for the preparation of compound 6. Isotopic purities of the tetra- and hexadeuterated linoleate isomers (Table 1) prepared from the triphenylphosphonium salts (Fig. 2) ranged from 96% for the tetradeuterated to 88% for the hexadeuterated linoleate isomers.

We found *Vernonia* and *Crepis* seed oils to be useful precursors in the preparation of multi-gram quantities of deuterium-labelled methyl linoleate and methyl linoleate isomers. *Crepis alpina* has been the most useful as precursor for the rapid and high-yield preparation of methyl *cis*-9,*cis*-12- and *trans*-9,*cis*-12-octadecadienoate-12,13-d_2.

ACKNOWLEDGMENTS

R. Kleiman and Dr. K. Carlson (USDA National Center for Agricultural Utilization Research, Peoria, IL) provided samples of *Crepis alpina* and *Vernonia galamensis*, respectively; Dr. F. Ayorinde (Howard University, Washington, DC) *Vernonia galamensis* oil and S. Duval (National Center for Agricultural Utilization Research, Peoria, IL) mass spectrometry.

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[Received September 21, 1990; accepted February 20, 1991]