The Lindlar-Catalyzed Reduction of Methyl Santalbate: A Facile Preparation of Methyl 9-*cis*,11-*trans*-Octadecadienoate-9,10-d_2

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ABSTRACT: Conjugated linoleic acid (CLA) has been associated with the reduction of chemically induced cancers in mice and rats and the suppression of atherosclerosis in rats. We have found seed oils to be a valuable source of precursors for the rapid preparation of gram quantities of deuterium-labeled fats. Methyl santalbate (methyl 11-*trans*-octadecen-9-ynoate), obtained from *Santalum album* (Linn.) seed, was reduced with Lindlar catalyst, quinoline, and deuterium gas to produce, in yields of 65–75%, the gram quantities of methyl 9-*cis*,11-*trans*-octadecadienoate-9,10-d_2 (CLA-d_2) we required for metabolism and oxidation studies. Unlike monoacetylenic and methylene-interrupted polyacetylenic fatty acid methyl esters, the conjugated system was reduced with no noticeable break in the rate of deuterium uptake. The quantity of poison (quinoline) present did influence the amount of CLA-d_2 produced, but the production of overreduced fatty acid methyl esters (perhaps because of the conjugated system) could not be prevented. Fractionation of the reaction mixture by silver resin chromatography resulted in the isolation of >99% chemically pure CLA-d_2 in yields of 60–70%.

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Conjugated linoleic acid (CLA; 9-*cis*,11-*trans*-octadecadienoic acid) has been associated with the reduction of chemically induced cancers in mice and rats and the suppression of atherosclerosis in rats (1,2). While commercially available samples of CLA (health-food stores and *via* the Internet) tend to contain a mixture of isomers (3), the 9-*cis*,11-*trans*-isomer is considered to be the active constituent (4). As part of our research into the metabolism and oxidation of CLA, we required CLA labeled with deuterium atoms on the 9- and 10-carbon atoms. Deuterium-labeled fats can be safely used in humans and their metabolism followed by gas chromatography/mass spectrometry (5,6).

Most synthetic schemes to produce deuterium-labeled fats involve multiple steps, require tedious purification procedures, and result in overall yields of only 5–15% (7,8). Seed oils are a valuable source of precursors for the rapid preparation of multigram quantities of deuterium-labeled fats (9,10). We found that santalbic acid (11-*trans*-octadecen-9-ynoic acid; also known as ximenynic acid), obtained from *Santalum album* seed oil, could be reduced with Lindlar catalyst and deuterium gas to prepare CLA-9,10-d_2 in gram quantities. Methyl santalbate may also be prepared from ricinoleic acid (12-hydroxy-9-*cis*-octadecenoic acid) as previously described (11).

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EXPERIMENTAL PROCEDURES

Materials. Santalum album seeds were obtained from Mr. B. Phillips (NCAUR, Peoria, IL). Quinoline and Lindlar catalyst were obtained from Aldrich (Milwaukee, WI). Celite™ 545 was obtained from J.T. Baker, Inc. (Phillipsburg, NJ). The benzene used (Fisher Scientific, Fair Lawn, NJ) was stored over molecular sieve 4A. Deuterium gas (98%) was obtained from Airco (Murray Hill, NJ). All other reagents were analytical grade or better.

Equipment. Fatty acid methyl esters (FAME) were analyzed with a Varian 3400 GC (Varian Instruments, Palo Alto, CA) equipped with a 30 m × 0.32 mm SP2380 (Supelco, Inc., Bellefonte, PA) capillary column and flame-ionization detector (FID). Helium was utilized as carrier gas. Methyl ester peaks were identified by comparison with standard FAME mixtures of known composition.

Gas chromatography/mass spectrometry (GC/MS) was used to determine the isotopic purity of the reduced FAME. Analyses were made on a Hewlett-Packard (Palo Alto, CA) Model 5889 GC/MS (quadrapole; positive chemical ionization mode; isobutane as ionizing gas) equipped with a 30 m × 0.25 mm Supelcowax 10 fused-silica capillary column (Supelco, Inc.). Data collection and manipulation have been described previously (12).

The C_{18} reversed-phase high-performance liquid chromatography (HPLC) system consisted of a 50 mm × 250 mm stainless steel column (5 micron particle size; Serva Feinbiochemica, Heidelberg, Germany) and a Rheodyne 7125 injector (Rheodyne, Inc., Cotati, CA) with a 2-mL injection loop. Both the silver resin and the C_{18} reversed-phase systems utilized Waters 510 HPLC pumps and R403 refractive index detectors (both Waters Associates, Milford, MA).

Silver resin chromatography for preparative separation of CLA isomers was done on a 2.5 × 45 cm glass column packed

TABLE 1
Composition of CLA-d_2 Reaction Mixtures[a]

Run #	Conditions	18:0-d_6 (%)	*trans*-13-18:1-d_4 (%)	*cis*-9 18:1-d_4 (%)	CLA-d_2 (%)	MeSan[a] (%)
1	a,b[b]	0.7	51.8	34.5	13.5	Trace[c]
2	a	—	6.8	9.0	76.2	7.8
3	a,c	—	2.5	6.8	63.2	27.5
4	a,c,d	—	3.5	8.3	74.0	14.2
5	e,f	—	38.8	23.0	38.2	
5	e	—	34.3	49.9	15.8	
6	e,g	—	11.1	14.4	74.5	
6	e,h	—	21.0	23.6	55.4	

[a]CLA, conjugated linoleic acid; MeSan, methyl santalbate; Trace, <0.5%.
[b]a, 0.65 g MeSan, 4.5 mL hexane, 6.5 mg catalyst, 75 µL quinoline, stir at room temperature for 20 min. b, No quinoline added. c, 250 µL quinoline added. d, Stir for 15 min. e, 0.250 g MeSan, 20 mL hexane, 25 mg catalyst, 250 µL quinoline, stir at room temperature for 15 min. f, Stir for 10 min. g, Stir for 4 min. h, Stir for 8 min.

with 200/270 mesh, silver ion-saturated Rohm and Hass XN1010 macroreticular sulfonic acid resin (13). For silver ion incorporation and packing of the glass columns see Reference 14. Samples were applied by needle-tipped syringe through a septum to the top of the column and eluted with 100% methanol at a flow rate of 7 mL/min.

Procedures. Isolation of methyl santalbate. *Santalum album* seeds were crushed with a mortar and pestle and the oil was extracted with hexane. The solvent was removed and the residue chromatographed through silica gel (diethyl ether/ hexane, 10:90, vol/vol). The triglycerides (TAG) were converted to FAME by room temperature alkali-catalyzed transesterification (15), and the methyl santalbate (72% of FAME) was isolated (97.9% pure) by preparative reversed-phase chromatography (1.86 g FAME; acetonitrile at 7 mL/min as solvent). *Santalum album* seeds (5.83 g) yielded 1.86 g oil (72.8% santalbic acid by GC) from which 1.14 g of highly purified (>98%) material was isolated by reversed-phase HPLC.

Deuterium incorporation. Benzene was added to a heat-dried 50 mL round-bottomed flask and was degassed twice with D_2/house vacuum and magnetic stirring. Lindlar catalyst and the appropriate poison (*via* syringe) were added and the slurry was degassed three times with D_2/house vacuum. After stirring at room temperature (21°C) for 10 min, the substrate (in 1–2 mL benzene) was added *via* syringe and the system was again degassed three times. Stirring was started and the amount of deuterium gas uptake was measured (16). After the specified time, the slurry was filtered through Celite™ to remove the catalyst. The solution was transferred to a separatory funnel with diethyl ether and washed with 5% sulfuric acid and then water. The organic layer was separated and dried over sodium sulfate for 2 h. The drying agent was removed by vacuum filtration, and the solvents were evaporated on a rotary evaporator (see Table 1 for reagent quantities and reaction times). Initial purification of the CLA-d_2 was accomplished utilizing C_{18} reversed-phase HPLC. The *trans/trans* CLA isomeric by-product was removed by silver resin chromatography as described in the paragraph on Equipment, above. Purity of the isolated CLA-d_2 was >99%.

RESULTS

A typical gas chromatographic trace of a CLA-d_2 reaction mixture is given in Figure 1. Compound #1 (all as FAME) was found to correspond to octadecanoate-d_6; compound #2 to 11-*trans*-octadecenoate-d_4; compound #3 to 9-*cis*-octadecenoate-d_4; compound #4 to 9-*cis*, 11-*trans*-octadecadienoate-d_2; compound #5 to 9-*trans*, 11-*trans*-octadecadienoate-d_2; and compound #6 to unreacted methyl santalbate. Compound designations were determined by GC (comparison with known standards) and by GC/MS (molecular weights/fragmentation patterns). The positions of the double bonds in the monoenoic FAME are tentative. Table 1 lists the chemical composition of reaction mixtures for which solvent volumes, poison to substrate ratios, and stirring times have been varied. A 100 µL aliquot was removed during runs #5 and #6 for analysis by GC. The deuterium distributions of the compounds listed in Table 1 are presented in Table 2.

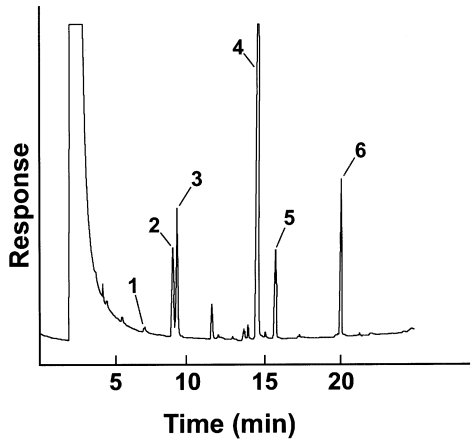

FIG. 1. Gas chromatographic analysis of CLA-d_2 fatty acid methyl ester reaction mixture using an SP2380 capillary column (see Experimental Procedures section for details). Peak #1 = 18:0; Peak #2 = 11-*trans*-18:1; Peak #3 = 9-*cis*-18:1; Peak #4 = 9-*cis*,11-*trans*-18:2; Peak #5 = 9-*trans*,11-*trans*-18:2; Peak #6 = 11-*trans*-octadecen-9-ynoate. CLA, conjugated linoleic acid.

TABLE 2
Deuterium Distributions from the Preparation of CLA-d_2

Run #	Peak #[a]	Deuterium distribution (%)									
		0	1	2	3	4	5	6	7	8	9
1	1	0.1	0.1	0.3	1.0	6.0	24.7	49.0	14.0	4.7	
1	2	0.1	0.5	2.0	16.8	65.7	12.0	2.0	0.6	0.3	0.1
1	3	0.1	0.5	3.3	17.4	59.6	15.9	2.2	0.6	0.3	0.1
1	4	1.1	10.2	82.5	3.8	1.9	0.4	0.1	0.1		
5	4	0.4	8.4	88.2	2.7	—	0.2				
6	4	0.5	8.6	87.8	2.8	—	0.2				

[a]1–4, Methyl santalbate reduction products: (#1) = 18:0-9,9,10,10,11,12-d_6; (#2) = *trans*-11-18:1-9,9,10,10-d_4; (#3)= *cis*-9-18:1-9,10,11,12-d_4; (#4) = CLA-9,10-d_2. For abbreviation see Table 1.

DISCUSSION

The Lindlar-catalyzed reduction (utilizing deuterium gas) of acetylenic precursors is a widely used and well-documented method (17,18) for the preparation of olefins labeled with deuterium atoms on the double bond(s).

While methyl santalbate (11-*trans*-octadecen-9-ynoate), obtained from *S. album* seed oil, could be readily reduced with Lindlar catalyst and deuterium gas to prepare methyl 9-*cis*, 11-*trans*-octadecadienoate-d_2 in gram quantities, the reaction yields and isotopic purities we obtained were lower than those values usually associated with this reaction (65–75%/82–88% vs. 94–97%/92–98%, respectively). Some 6–7% of the *trans/trans*-isomer was also generated during the reduction, an amount twice the 3–5% usually observed during reductions using Lindlar catalyst. By contrast, the reduction of methyl eicosatetraynoate (eicosatetra-5,8,11,14-ynoate) to 5-*cis*,8-*cis*,11-*cis*,14-*cis*-eicosatetraenoate-5,6,8,9,11,12,14,15-d_8 was accomplished in >90% chemical yield (<2% overreduction; 6% *trans*-isomers) and with an isotopic purity >93% (Adlof, R.O., unpublished results). Increasing the ratio of quinoline to substrate and the volume of solvent resulted in improved yields (chemical and isotopic) of the desired CLA-d_2 product. The rate of deuterium gas uptake also increased, and some reduction of the CLA-d_2 to monoenoic FAME-d_4's could not be prevented. Isotopic purities of the monoene-d_4's were significantly lower and found to contain substantial amounts (16–17%) of 18:1-d_3. These results often denote reduction *via* a different mechanistic pathway, and include hydrogen-deuterium (H-D) exchange on the carbon backbone of the substrate. A comparison may be made with the deuterium-scatter observed by Emken (19) when he analyzed the conjugated diene intermediates formed during the reduction of methyl linoleate (9-*cis*,12-*cis*-octadecadienoate) to methyl stearate (octadecanoate)-9,10,12,13-d_4 using Wilkinson's catalyst [tris-triphenylphosphinechlororhodium (I)] and deuterium gas.

The only drawback to this procedure is the difficulty in establishing an endpoint. Since deuterium gas uptake slowed but never stopped, no endpoint could be reliably determined. We found the best approach was to periodically remove aliquots of the reaction mixture for analysis by GC. The Lindlar-catalyzed reduction of methyl santalbate is not the preferred method to prepare nondeuterated CLA. Zinc in isopropyl alcohol yielded comparable results and other syntheses are available (20). But the Lindlar-catalyzed reduction of methyl santalbate with deuterium gas is the most useful method we have found to rapidly prepare, in 60–70% overall yields, multigram quantities of deuterium-labeled CLA with chemical purities >99% and isotopic purities of 85–88%.

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