SYNTHESES OF GEOMETRIC ISOMERS OF DI-, TETRA-, AND HEXADEUTERATED 12-OCTADECENOATES AND THEIR TRIGLYCERIDES

Henry Rakoff and Edward A. Emken
Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture,* Peoria, Illinois 61604

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SUMMARY

Four geometrically isomeric deuterated 12-octadecenoates and their triglycerides were synthesized for use in metabolism studies in humans. Methyl *trans*-12-octadecenoate-15,15,16,16-d_4 (81.7% d_4) and the corresponding *cis* isomer (88.9% d_4) were obtained by the Wittig reaction between hexyl-3,3,4,4-d_4-triphenylphosphonium bromide (or iodide) and methyl 12-oxododecanoate. The phosphonium salt was derived, in several steps, from 3-hexynol and the methyl 12-oxododecanoate from cyclododecene. Methyl *cis*-12-octadecenoate-9,10,15,15,16,16-d_6 (79.9% d_6) was obtained via the Wittig reaction between hexyl-d_4-triphenylphosphonium bromide and methyl 12-oxododecanoate-9,10-d_2. This deuterated aldehyde ester was prepared by lead tetraacetate oxidation of methyl 12,13-dihydroxyoctadecanoate-9,10-d_2 which was obtained, in several steps, from *Vernonia anthelmintica* seed oil. A simple and convenient stereospecific synthesis of methyl *trans*-12-octadecenoate-9,10-d_2 (84.9% d_2) was accomplished by thermal decomposition of methyl 12,13-di-O-(ethoxymethylene)-octadecanoate-9,10-d_2 which was prepared, in several steps, from 12,13-epoxy-9-octadecenoate obtained from *Vernonia* oil. All deuterations were catalyzed by tris(triphenylphosphine)chlororhodium. *Cis*- and *trans*- isomers were separated on an Amberlyst XN 1005 column impregnated with silver ions.

KEY WORDS: Deuterium labeling, Wittig reaction, *Vernonia anthelmintica* seed oil, Geometric isomers, Fatty esters.

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INTRODUCTION AND DISCUSSION

For continuation of our work on lipid metabolism in humans using deuterated triglycerides (1), methyl *trans*-12-octadecenoate-9,10-d_2, *1*, methyl *cis*- and *trans*-12-octadecenoate-15,15,16,16-d_4, *2*, and methyl *cis*-12-octadecenoate-9,10,15,15,16,16-d_6, *3*, and their triglycerides were synthesized. A summary of the mass spectral analyses of the various deuterated compounds prepared is presented in Table I.

TABLE I

Mass Analyses for Deuterium

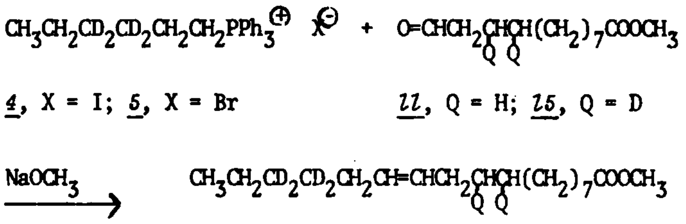
No.	Substance	Number of deuterium atoms[a] 0	1	2	3	4	5	6	7	Average no. per molecule
10	Bromohexane-2,2,3,3-d_4	0.1	0.1	1.8	17.6	79.6	0.9			3.80
10	Bromohexane-3,3,4,4-d_4	0.8	0.2	---	8.6	88.7	1.2	0.3		3.89
4	Iodohexane-3,3,4,4-d_4	0.3	0.3	0.5	3.2	94.1	1.2	0.2		3.96
15	12-Oxo-12:0-9,10-d_2	2.7	10.3	82.7	0.3	0.3				1.90
1	12*t*-18:1-9,10-d_2[b]	4.5	7.3	84.9	1.9	1.3				1.88
2	12*c*-18:1-15,15,16,16-d_4	1.1	0.4	2.5	3.5	88.9	1.7	0.4		3.87
2	12*t*-18:1-15,15,16,16-d_4	2.1	---	3.0	5.7	81.7	7.5			3.87
3	12*c*-18:1-9,10,15,15,16,16-d_6	---	---	---	---	2.8	10.9	79.9	6.4	5.90

[a] Values in table are in percent.

[b] Methyl *trans*-12-octadecenoate-9,10-d_2.

For the tetra- and hexadeuterated compounds, hexyl-3,3,4,4-d_4-triphenylphosphonium iodide, *4* (or bromide, *5*), was required for condensation with an appropriate aldehyde ester in a Wittig (2) reaction (Sequence 1). Of four possible synthetic routes investigated for preparation of the phosphonium salt starting with 3-hexynol, the most successful was the one (Sequence 2) we developed for protection of the hydroxy group by forming the tetrahydropyranyl ether, *6*. This ether was deuterated catalytically with tris(triphenylphosphine)chlororhodium, $(Ph_3P)_3RhCl$, (3) to the tetradeutero ether, *7*, which, in turn, was transformed into the tetradeutero iodide, *8*, by treatment with sodium iodide in 95%

SEQUENCE 1



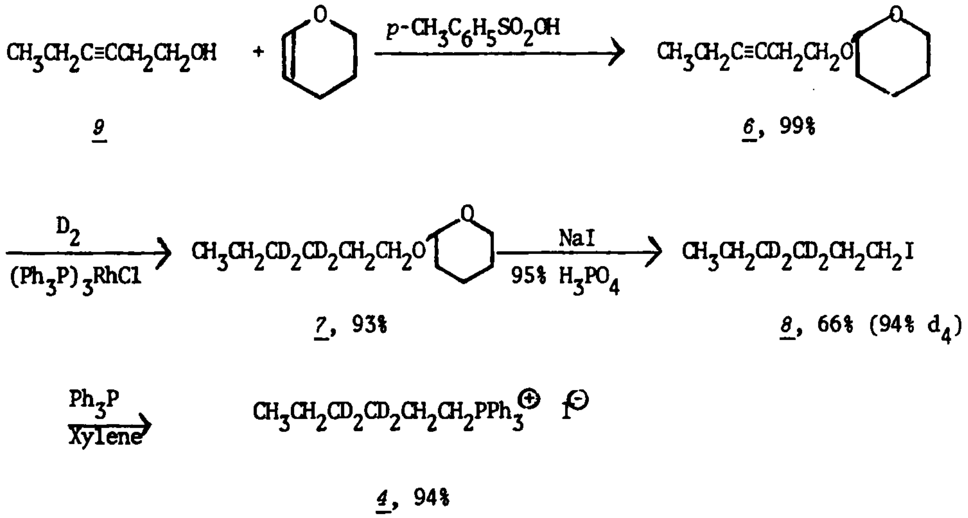
$$CH_3CH_2CD_2CD_2CH_2CH_2PPh_3^{\oplus}\ X^{\ominus} + O{=}CHCH_2\underset{Q}{C}H\underset{Q}{C}H(CH_2)_7COOCH_3$$

4, X = I; 5, X = Br　　11, Q = H; 15, Q = D

$$\xrightarrow{NaOCH_3} CH_3CH_2CD_2CD_2CH_2CH{=}CHCH_2\underset{Q}{C}H\underset{Q}{C}H(CH_2)_7COOCH_3$$

Product	Phosphonium salt	Q	Yield	D Content
2	5	H	81%	81.7% d_4
2	4	H	71%	88.9% d_4
3	5	D	77%	79.9% d_6

SEQUENCE 2



phosphoric acid according to the method of Stone and Shechter (4). The iodohexane-d_4, *8*, analyzed 94.1% d_4. Reaction with triphenylphosphine in xylene (5) converted *8* into hexyl-3,3,4,4-d_4-triphenylphosphonium iodide, *4*.

The hydroxy hydrogen of 3-hexynol, *9*, is replaceable and was responsible for loss of label in the other methods attempted. Thus, when *9* was deuterated directly and then converted to the bromohexane-d_4, *10*, by treatment with triphenylphosphine and bromine (6), the product contained 79.6% d_4. Replacement of the hydroxy hydrogen in *9* with deuterium before catalytic deuteration (Sequence 3) permitted recovery of *10* containing 88.7% d_4. This was converted to the phosphonium salt *5* as described for the preparation of *4*. Conversion of *9* to 1-bromo-3-hexyne also was made but, unfortunately, this compound was not reduced completely to *10* by the $(Ph_3P)_3RhCl$ catalyst.

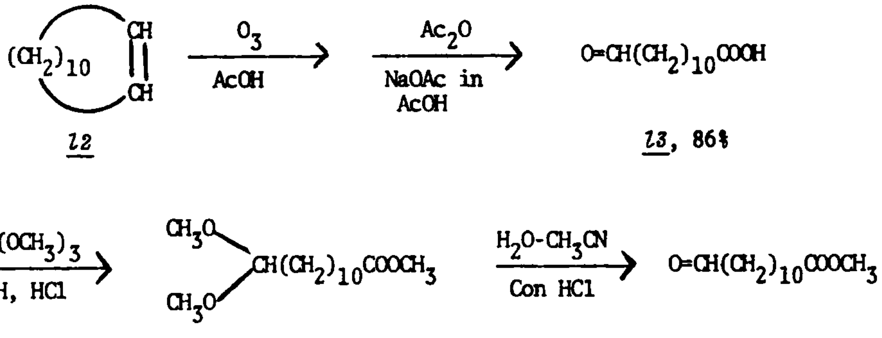
SEQUENCE 3

$$\underset{\textit{9}}{CH_3CH_2C{\equiv}CCH_2CH_2OH} \xrightarrow[DCl]{D_2O} \xrightarrow[(Ph_3P)_3RhCl]{D_2} \underset{\textit{20},\ 65\%}{CH_3CH_2CD_2CD_2CH_2CH_2OD}$$

$$\xrightarrow[Br_2]{Ph_3P} \underset{\textit{10},\ 81\%\ (88.7\%\ d_4)}{CH_3CH_2CD_2CD_2CH_2CH_2Br} \xrightarrow[Xylene]{Ph_3P} \underset{\textit{5},\ 91\%}{CH_3CH_2CD_2CD_2CH_2CH_2PPh_3^{\oplus}Br^{\ominus}}$$

For the preparation of methyl *trans*-12-octadecenoate-15,15,16,16-d_4, *2*, methyl 12-oxododecanoate, *11*, was required. It was prepared (Sequence 4) by ozonizing cyclododecene, *12*, in 86% yield to 12-oxododecanoic acid, *13*, by the method of Siclari (7). Reaction at room temperature with methanol and trimethyl orthoformate (8) gave the acetal ester, *14*, in 69% yield. Compound *14* was then converted in 95% yield to the aldehyde ester, *11*, by concentrated HCl in acetonitrile-water solution (8).

SEQUENCE 4



12

13, 86%

14, 69%

11, 95%

The two fragments, *5* and *11*, were condensed in the presence of sodium methoxide in a Wittig reaction (2,6,9,10,11) to yield the desired product, *2*, in 81% yield. This product contained 81.7% d_4. With *4* and *11* the yield of *2* was 71% and the isotopic content was increased to 88.9% d_4.

It is customary to run the Wittig reaction overnight (2,6,9,10). We have determined that for these compounds, the Wittig reaction is essentially complete 5 min after completion of the addition of the aldehyde ester to the phosphorane at room temperature. Further, the success of the Wittig reaction depends on the quality of the sodium methoxide. We used Harshaw sodium methoxide, and when we stored portions of it in small vials in a desiccator, we were able to obtain consistently good results. It is well to check the quality of the base on a small scale before committing large amounts of intermediates to it. If addition of the dimethylformamide solution of the phosphonium salt to the sodium methoxide does not produce a deep orange color, the base is not good and poor yields will result.

Both the *cis* and *trans* isomers of 2 were required for the human metabolism studies. Product 2, as obtained from the Wittig reaction, contained some *trans* isomer. Separation of *trans* from *cis* was accomplished by passage in methanol through a macroreticular resin (Amberlyst XN 1005) impregnated with silver ions (12), and the *cis* and *trans* isomers were isolated in the ratio of 6.3:1 (i.e., 86% *cis*). To prepare larger quantities of the *trans* isomer, the *cis* isomer was isomerized with nitrous acid according to the method of Litchfield *et al.* (13), and the isomers were separated on the silver resin column. In this case the *cis* and *trans* isomers were obtained in the ratio of 1:4.2 (i.e., 81% *trans*).

Methyl *cis*-12-octadecenoate-9,10,15,15,16,16-d_6, 79.9% d_6, 3, was prepared in 77% yield by a Wittig reaction between 5 and methyl 12-oxododecanoate-9,10-d_2, 15. This deuterated aldehyde ester, 15, was obtained (Sequence 5) in approximately 60% yield by the oxidation of

SEQUENCE 5

Vernonia anthelmintica seed oil $\xrightarrow[\text{3. } CH_3OH,\ H_2SO_4]{\text{1. AcOH} \quad \text{2. KOH, } CH_3OH}$ $CH_3(CH_2)_4CHOHCHOHCH_2CH{=}CH(CH_2)_7COOCH_3$

17

$\xrightarrow[(Ph_3P)_3RhCl]{D_2}$ $CH_3(CH_2)_4CHOHCHOHCH_2CHDCHD(CH_2)_7COOCH_3$

16

$\xrightarrow[AcOH]{Pb_3O_4}$ $O{=}CHCH_2CHDCHD(CH_2)_7COOCH_3$

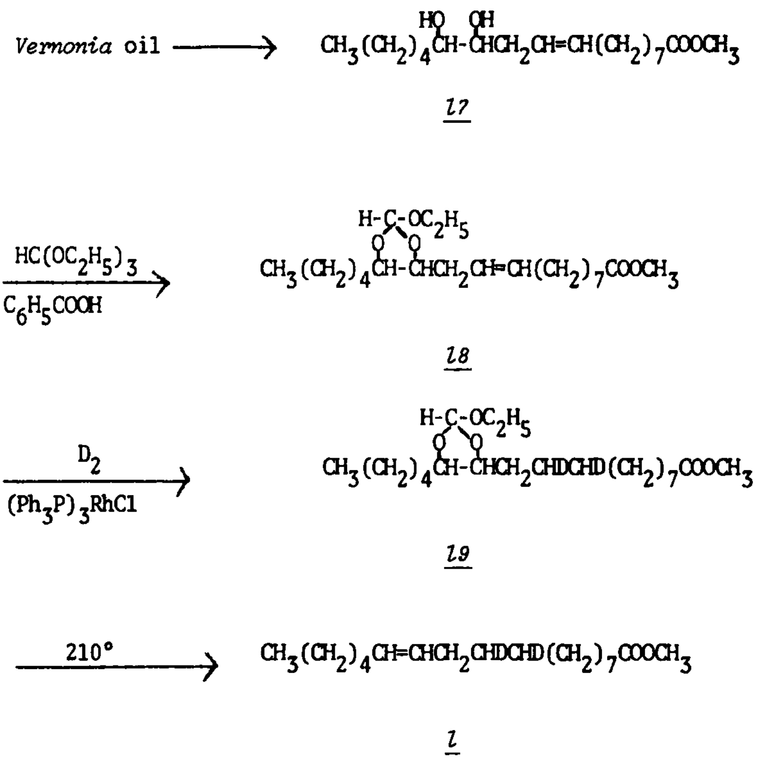
15

methyl 12,13-dihydroxyoctadecanoate-9,10-d_2, *16*, by red lead (Pb_3O_4) and glacial acetic acid according to the method of Scanlan and Swern (14). Cleavage of the *vicinal* glycol also was accomplished successfully with periodic acid (15). However, distillation of the product resulted in formation of the cyclic trimer in the receiver, and this method was abandoned in favor of the lead tetraacetate procedure.

The dihydroxyester, *16*, was obtained from *Vernonia anthelmintica* seed oil by modifications of the method of Gunstone (16) and of Maerker *et al.* (17). *Vernonia* oil has the following approximate composition (18): 3% palmitate, 1.5% stearate, 3.9% oleate, 11.8% linoleate, and 78% vernolate (12,13-epoxy-9-octadecenoate). The epoxide ring was opened with glacial acetic acid to give the hydroxy acetoxy glyceride which was saponified to the dihydroxy acid by KOH in methanol and then converted to the esters with methanol and sulfuric acid. The unsaturated *Vernonia* esters were deuterated with deuterium and $(Ph_3P)_3RhCl$ as catalyst. The reduced *Vernonia* esters consisted of dihydroxystearate-d_2, stearate-d_4 (from the linoleate), stearate-d_2 (from the oleate), and undeuterated saturated esters (from the original oil). This mixture was used to prepare *15*, and the saturates were not removed until purification on the silver resin column. Compound *3* (79.9% d_6) was separated into its geometric isomers (*cis*:*trans* = 7.5:1, i.e., 88% *cis*) on the silver resin column.

Methyl *trans*-12-octadecenoate-9,10-d_2 (84.9% d_2, *1*) was obtained from the mixed *Vernonia* methyl esters (Sequence 6). Methyl *threo*-12,13-dihydroxy-9-octadecenoate, *17*, present in the mixture, was converted to the 12,13-di-O-(ethoxymethylene)-9-octadecenoate, *18*, as previously described (19) by the method of Crank and Eastwood (20). This compound was catalytically deuterated to yield 12,13-di-O-(ethoxymethylene)-octadecanoate-9,10-d_2, *19*, which was converted by heating to *1*. The geometric isomers were separated (*trans*:*cis* = 12.8:1, i.e., 93% *trans*) on the silver resin column to give the desired *trans* isomer in 60% overall yield from the mixed *Vernonia* esters. Double bond location by ozonolysis confirmed the location of the unsaturation.

SEQUENCE 6



Vernonia oil $\longrightarrow$ $CH_3(CH_2)_4\overset{HO}{C}H-\overset{OH}{C}HCH_2CH{=}CH(CH_2)_7COOCH_3$

17

$\xrightarrow[C_6H_5COOH]{HC(OC_2H_5)_3}$ $\overset{H-\underset{O\;\;\;O}{C}-OC_2H_5}{CH_3(CH_2)_4CH-CHCH_2CH{=}CH(CH_2)_7COOCH_3}$

18

$\xrightarrow[(Ph_3P)_3RhCl]{D_2}$ $\overset{H-\underset{O\;\;\;O}{C}-OC_2H_5}{CH_3(CH_2)_4CH-CHCH_2CHDCHD(CH_2)_7COOCH_3}$

19

$\xrightarrow{210°}$ $CH_3(CH_2)_4CH{=}CHCH_2CHDCHD(CH_2)_7COOCH_3$

1

For the metabolic studies glycerides, rather than methyl esters, were needed. The methyl esters were saponified to the free acids which were treated with glycerol and *p*-toluenesulfonic acid at 100° in a modification of the procedure of Wheeler *et al.* (21). The reaction was monitored by TLC; when TLC analysis showed the presence only of triglyceride and free fatty acid (no mono- or diglycerides), the reaction was stopped and triglycerides were isolated by column chromatography on Florisil using isooctane and isooctane:ethyl ether = 90:10 as eluants. Yields of triglycerides were 82-88%.

EXPERIMENTAL

Instruments: Mass spectra were determined on a Nuclide 12-90G spectrometer with 70 electron volt ionization inlet, and NMR spectra were obtained on a Varian HA 100 MHz spectrometer. Gas-liquid chromatography was carried out with an F&M Model 720 gas chromatograph equipped with a thermal conductivity detector, operated at a helium flow rate of 65 ml/min at 70 psi. The columns used included 4 ft x 1/4 in. 10% EGSS-X and 10 ft x 1/4 in. 15% EGSS-X (both on 100/120 mesh Gas Chrom P) and 12 ft x 1/4 in. 3% JXR on 100/120 mesh Gas Chrom Q. Thin-layer chromatography was conducted on 0.25 mm precoated TLC silica gel F254 plates (E. Merck).

Reagents: tris(Triphenylphosphine)chlororhodium from Eastman or Strem Chemical Company; 3-hexynol from Farchan Division, Storey Chemical Company; sodium methoxide from Harshaw Chemical Company; triphenylphosphine, cyclododecene, and dihydropyran from Aldrich Chemical Company.

Deuterations with tris(triphenylphosphine)chlororhodium (3)

The apparatus consisted of a 2-liter 3-necked flask equipped with a very efficient magnetic stirrer and an inlet adapter which introduced gas above the liquid. The flask was connected to a manometric apparatus (22) which maintained one atmosphere pressure within the system and with which one could measure the volume of gas absorbed. Benzene (950 ml) was placed in the flask and oxygen, to which the catalyst is sensitive, was removed by rapid stirring and alternately evacuating the system and flushing with N_2(4X) and with D_2 (2X). Stirring was stopped, the tris(triphenylphosphine)chlororhodium, $(Ph_3P)_3RhCl$, catalyst was added, and the system was evacuated and flushed with D_2 (2X). Stirring was resumed and D_2 uptake by the catalyst was monitored until it was complete. Stirring was stopped, the compound to be deuterated was added, and the system was again evacuated and flushed (2X) with D_2. Stirring was started and continued until no more D_2 was taken up (4-8 hr). When reduction is proceeding, the solution is the color of iced tea; if stirring is inefficient, the solution will be deep red in color.

2-Tetrahydropyranyl 3-hexynyl ether, *6*. In a 500-ml, 3-necked flask equipped with a mechanical stirrer and a thermometer were placed 3-hexynol (49 g, 0.5 mol) and *p*-toluenesulfonic acid (1.9 g, 0.01 mol). While a slow stream of N_2 was maintained through the apparatus, dihydropyran (50.4 g, 0.6 mol) was added over 15 min while the temperature was maintained at 20-37° by intermittent cooling. After 2 hr the reaction mixture was passed through a column (30 mm I.D.) of silica gel (100 g) slurried in petroleum ether. The eluate was evaporated on a rotary evaporator to yield 2-tetrahydropyranyl 3-hexynyl ether, *6* (90.25 g, 99% yield, 92% pure by GLC).

2-Tetrahydropyranyl hexyl-3,3,4,4-d_4 ether, *7*. For this reduction, 10 g of rhodium catalyst in 950 ml of benzene was used with 90.25 g of *6* as described above. The benzene was removed on a rotary evaporator to obtain reddish brown material (119 g) which was passed through a column of silica gel to remove catalyst and color. The petroleum ether eluate was removed on a rotary evaporator to leave 2-tetrahydropyranyl hexyl-3,3,4,4-d_4 ether, *7* (87.6 g, 93% yield, 98% pure by GLC).

Iodohexane-3,3,4,4-d_4, *8*, (4). A 500-ml, 3-necked flask was equipped with a mechanical stirrer, a thermometer, a dropping funnel, a condenser, and a means for passing N_2 through the flask. Into this flask were placed 85% H_3PO_4 (126.2 g) and P_2O_5 (28.82 g) (equivalent to 1.5 moles of 95% H_3PO_4). The temperature rose to 80° and the flask was cooled to room temperature with an ice bath. To the flask was added NaI (150 g, 1 mol), with stirring, whereupon the contents became yellow and sludgy. Then, 2-tetrahydropyranyl hexyl-3,3,4,4-d_4 ether, *7* (47.5 g, 0.25 mol), was added dropwise over 20 min while the temperature rose from 17 to 34°. The flask contents at this point were purple and sludgy. GLC analysis on 15% EGSS-X at 101° of a sample showed that the ether had been converted to the alcohol. The reaction vessel was heated at 120° for 2 hr, at which point GLC analysis showed the presence of iodohexane and the absence of hexanol and THP ether. The flask contents were cooled, diluted with H_2O, and extracted into ethyl ether. The ether extract was washed with 50% $Na_2S_2O_3$ and with saturated NaCl solution and dried over Na_2SO_4. Removal of the drying agent and solvent left a black liquid (75 g) which was distilled to give iodohexane-3,3,4,4-d_4, *8* (35.9 g, 66.5% yield), b.p. 92-94°/38-43 mm. Mass spectral analysis indicated 94.1% d_4.

Hexyl-3,3,4,4-d_4-triphenylphosphonium iodide, *4*, (5). In a 500-ml round-bottomed flask equipped with a reflux condenser were placed *8*, iodohexane-3,3,4,4-d_4 (35.5 g, 0.16 mol), triphenylphosphine (50 g, 0.19 mol), and xylene (150 ml). The flask was heated at reflux for 2 hr and the disappearance of *8* was followed by GLC on 3% JXR at 53°. The hot flask contents were poured into a beaker and, when cool, the xylene was decanted. The taffee-like product was triturated (5X) with fresh portions of ethyl ether, and the white solid obtained was filtered and washed several times with ether. The product was dried to constant weight in a vacuum desiccator to yield 74 g (94% yield) of white phosphonium salt *4*.

Hexanol-3,3,4,4-d_4, *20*. 3-Hexynol (50 g, 0.51 mol) was shaken vigorously three times with separate portions of 99.83% D_2O (100 ml) containing DCl (1N in D_2O). The deuterium exchanged 3-hexynol was deuterated as described above using 5 g of $(Ph_3P)_3RhCl$ catalyst in 950 ml of benzene for 35 g of alkynol. After removal of the benzene, petroleum ether (100 ml) was added to precipitate the catalyst and the brownish spent catalyst (4 g) was removed by filtration. The petroleum ether was removed on a rotary evaporator to give a black liquid (33.94 g) which was distilled under reduced pressure. The main fraction (27.12 g) distilled at 85-89°/44 mm.

Bromohexane-3,3,4,4-d_4, *10*, (6). In a 1-liter, 3-necked flask equipped with a mechanical stirrer, a thermometer, a nitrogen inlet and outlet, and a 25-ml burette was placed triphenylphosphine (110 g, 0.42 mol) dissolved in dimethylformamide (300 ml) dried over molecular sieve 3A. To this was added *20*, hexanol-3,3,4,4-d_4 (42.3 g, 0.4 mol). Bromine (21 ml, 65.5 g, 0.41 mol) was added over 40 min while the temperature was maintained at 40-45° by rate of addition and external cooling. When the theoretical amount of bromine has been added, the color imparted by the bromine persists, so that it acts as its own indicator. The reaction mixture was distilled at 0.1 mm and a maximum pot temperature of 90° through a Dry-Ice-cooled condenser into a Dry-Ice-cooled receiver. The clear distillate was diluted with water and extracted into petroleum ether. The PE extract was washed with H_2O and dried over Na_2SO_4. Removal of the solvent on the rotary evaporator gave a clear liquid (57.44 g) which, on distillation, gave *10* (54.8 g, 81% yield) boiling at 65-67°/33 mm. Analysis by mass spectrometry showed 88.7% d_4.

Hexyl-3,3,4,4-d_4-triphenylphosphonium bromide, *5*. Bromohexane-d_4, *10*, was converted to *5* in 91.5% yield as described above for the preparation of the phosphonium iodide, *4*.

12-Oxododecanoic acid, *13*, (7). In a 1-liter, 3-necked flask equipped with a sparger, a mechanical stirrer, a low temperature thermometer, and a reflux condenser were placed cyclododecene (76 g, 0.43 mol) and glacial acetic acid (66.6 ml, 70 g, 1.16 mol). Sufficient cyclohexane was added to fill the flask almost up to the necks. The Welsbach Ozone Generator was set at 3 psi, 115 volts, SCF 0.08. The reaction vessel was cooled in an ice bath to 5°. Ozone was passed through the reaction mixture for about 4 hr until ozone could be detected, by means of starchiodide paper, exiting from the reflux condenser. After the ozone flow was stopped, the system was flushed with N_2 for 5 min and the cyclohexane was decanted. Acetic anhydride (100 g) was added to the viscous mass and with stirring the mixture was allowed to warm to 15°. Then there was added, very carefully and in small portions, 37 g of a solution of sodium acetate (10 g) in glacial acetic acid (90 g). This reaction is exothermic but it has an induction period, so care must be exercised. The temperature was maintained at 25-30° by rate of addition and by cooling with an ice bath. After all the sodium acetate solution had been added, the reaction mixture (334 g) was maintained in a N_2 atmosphere in a H_2O bath at 35° for 1 hr and then was concentrated on a rotary evaporator. To the product (234 g) was added H_2O (200 ml) and the mixture was boiled with stirring for 0.5 hr in a N_2 atmosphere. On cooling with stirring, there was formed a creamy white precipitate which was collected by filtration and pumped to constant weight in a vacuum desiccator over $CaCl_2$. There was obtained 13 (80 g, 86% yield), m.p. 64-67°. Literature (7) m.p. is 65-68°.

Dimethyl acetal of methyl 12-oxododecanoate, 14, (8). 12-Oxododecanoic acid, 13, (69.1 g, 0.32 mol) was dissolved in methanol (200 ml) in a 500-ml round-bottomed flask. To this was added trimethyl orthoformate (75 ml, 72.75 g, 0.686 mol) and concentrated HCl (10 drops). The reaction was monitored by TLC using the solvent system hexane:ethyl ether:acetic acid = 30:70:1. After 3.5 hr, H_2O (200 ml) was added and the reaction mixture was extracted into petroleum ether. The combined PE extracts were washed with H_2O and dried over anhydrous Na_2SO_4. The PE was removed on a rotary evaporator to yield a yellow oil (76.6 g) which was distilled under reduced pressure to give 14 (60.38 g, 69% yield), b.p. 140-148°/0.3 mm.

Methyl 12-oxododecanoate, *11*, (8). The acetal ester *14* (50 g, 0.18 mol) was dissolved in H_2O (25 ml) and sufficient acetonitrile (275 ml) to make a homogeneous solution. GLC on 3% JXR at 172° showed the presence of three components, the acetal ester *14*, the aldehyde ester *11*, and an enol ether, $CH_3O\text{-}CH{=}CH\text{-}(CH_2)_9COOCH_3$. Concentrated HCl (15 drops) was added and 16 hr later complete hydrolysis was demonstrated by GLC on 3% JXR. Water was added and the product was extracted into petroleum ether. The combined PE extracts were washed with H_2O and dried over anhydrous Na_2SO_4. Removal of the drying agent and solvent left a clear liquid (44.45 g) which was distilled to yield *11* (39.73 g, 95% yield) b.p. 110-120°/0.2 mm.

Methyl 12,13-dihydroxy-9-octadecenoate, *17*. *Vernonia anthelmintica* seed oil contains approximately 78% of 12,13-epoxy-9-octadecenoate (18). The *Vernonia* oil was acetolyzed, saponified, and esterified, by a slight modification of the methods of Gunstone (16) and of Maerker *et al.* (17), to mixed *Vernonia* methyl esters. GLC of this mixture on 10% EGSS-X at 195° showed only palmitate, stearate, oleate, and linoleate. A sample of the reaction mixture was treated with acetone and $BF_3 \cdot MeOH$ (23) to form the dioxolane of *17* which then showed up on the GLC as an additional later peak comprising about 78% of the total areas under all the peaks.

Methyl 12,13-dihydroxyoctadecanoate-9,10-d_2, *16*. For this reduction, 10 g of rhodium catalyst in 950 ml of benzene was used with 198 g of mixed *Vernonia* esters as described above. To the thick, reddish liquid (351 g) remaining after removal of the benzene, 95% ethanol (350 ml) was added and the mixture was heated to boiling and filtered to remove spent catalyst (about 7.7 g). On cooling in an ice bath, the dark ethanol solution deposited a first crop (75.5 g) of crystals. A second crop (18 g) was obtained by cooling in an ice bath, and a third crop (15.5 g) by cooling to -20° in a freezer. The ethanol solution was concentrated on a rotary evaporator to about 125 ml and cooled again in ice to give a fourth crop (14.8 g). Thus, 124 g of *16* was recovered. Each of these fractions showed only palmitate and stearate on GLC on 10% EGSS-X at 198°. A sample was treated with acetone and $BF_3 \cdot MeOH$ (23) to form the dioxolane of the *vicinal* diol which showed up as a large later peak. This saturated dioxolane has a shorter retention time than the dioxolane of the undeuterated *Vernonia* esters.

Methyl 12-oxododecanoate-9,10-d_2, *15*, (14). In a 1-liter, 3-necked round-bottomed flask equipped with a mechanical stirrer and a thermometer were placed *16* (and other deuterated *Vernonia* esters) (75.12 g) and glacial acetic acid (500 ml). The flask was heated to 57° and Pb_3O_4 was added in portions. As each portion was added, the reaction mixture became cloudy and orange but cleared up in 1 to 2 min. A total of 179 g of Pb_3O_4 was added over 50 min. Presence of excess oxidant can be detected by wet starch-potassium iodide paper. The temperature was maintained at 57-63° by controlling the rate of addition. The cooled reaction mixture was diluted, in two portions, with H_2O (1800 ml), and each portion was extracted with ethyl ether (3 X 150 ml). The combined ether extracts were washed several times with saturated $NaHCO_3$ solution and with H_2O and dried over anhydrous Na_2SO_4. After removal of the drying agent and ether, the residual liquid (64.8 g) was distilled under reduced pressure to yield a main fraction (33.2 g) consisting principally of *15*, methyl 12-oxododecanoate-9,10-d_2, b.p. 105-125°/0.14 mm. This compound analyzed 82.7% d_2. The next fraction (8.11 g), b.p. 126-153°/0.14 mm contained approximately equal amounts of *15* and methyl stearates. If one assumes the starting material to contain 78% of *16*, the percentage yield of *15* based on the main fraction alone is 82%.

Methyl 12-octadecenoate-15,15,16,16-d_4, 2. In a 1-liter, 3-necked round-bottomed flask equipped with a mechanical stirrer, a thermometer, a dropping funnel, and a N_2 inlet and outlet was placed sodium methoxide (9.47 g, 0.175 mol). To this was added over 13 min a solution of 5, hexyl-3,3,4,4-d_4-triphenylphosphonium bromide (86 g, 0.2 mol) dissolved in dimethylformamide (200 ml) which had been stored over molecular sieve 3A. No heat was evolved but the solution became cloudy and deep orange in color. One hour later, 11, methyl 12-oxododecanoate (32 g, 0.14 mol) dissolved in DMF (100 ml) was added over 15 min. The temperature was maintained at 30-35° by rate of addition and by an external H_2O bath, The reaction mixture was analyzed by GLC on 10% EGSS-X at 172° 5 min after completion of the addition. Oleate was present and aldehyde ester was absent. The color of the mixture had faded to a pale yellow. This material was diluted with H_2O (500 ml), cooled, and extracted with petroleum ether (3 X 250 ml). The combined PE extracts were washed with H_2O (2 X 250 ml) and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent left an oil (43.89 g) which was distilled to give 2 (34.4 g, 81.7% yield), b.p. 120-140°/0.14 mm. Mass spectral analysis indicated this material was 81.7% d_4. The *cis* and *trans* isomers were obtained from the silver resin column in the ratio of 6.3:1, i.e., 86% *cis*.

In a similar manner, 4 and 11 gave 2 in 70% yield. Mass spectral analysis of 2 indicated 88.9% d_4.

Methyl 12-octadecenoate-9,10,15,15,16,16-d_6, 3. Hexyl-d_4-triphenylphosphonium bromide, 5, and methyl 12-oxododecanoate-9,10-d_2, 15, were condensed in the presence of sodium methoxide as described for the preparation of 2 to yield methyl 12-octadecenoate-9,10,15,15,16,16-d_6, 3 (33.8 g, 77.3% yield) b.p. 130-145°/0.16 mm. This material contains some saturated esters derived from *Vernonia* oil. Separation of the saturated and unsaturated compounds was effected on the silver resin column, and the *cis* and *trans* isomers of 3 were obtained in the ratio of 7.5:1, i.e., 88% *cis*. The product analyzed 79.9% d_6.

Methyl *trans*-12-octadecenoate-9,10-d_2, *1*, (19,20). In a 250-ml round-bottomed flask attached to a condenser set for distillation were placed *Vernonia* esters (52 g), triethyl orthoformate (18 g, 0.12 mol), and benzoic acid (0.03 g). The flask was heated in an oil bath to and maintained at 120° for 1 hr during which time ethanol (13.3 ml, 0.227 mol) was collected. The reaction mixture was cooled and ethyl ether and solid Na_2CO_3 were added. After removal of the Na_2CO_3 and ether, there remained an amber oil (57.25 g) containing, among other things, methyl 12,13-di-O-(ethoxymethylene)-9-octadecenoate, *18*.

A portion (50.12 g) of the mixture containing *18* was treated in 950 ml of benzene with D_2 in the presence of the rhodium catalyst (5 g) as described above. Removal of the benzene left a thick red liquid (61.6 g) which was diluted with petroleum ether (100 ml), filtered to remove spent catalyst (3.16 g), and passed through a column (30 mm I.D.) containing silica gel (40 g). The product (44.7 g) obtained after evaporation of 600 ml of petroleum ether eluate was heated slowly in an oil bath to a maximum temperature of 225° and distillate was collected. The black reaction mixture was passed through a column (22 mm I.D.) packed with silica gel (10 g) to remove some of the black material. The product (34.63 g) obtained by elution with petroleum ether (150 ml) was separated on the silver resin column to give a saturated fraction (6 g), an intermediate fraction (3.92 g), methyl *cis*-12 octadecenoate-9-10-d_2 (1.61 g) and methyl *trans*-12-octadecenoate-9,10-d_2, *1* (20.58 g). The *cis/trans* ratio was 1:12.8 or 93% *trans*. Mass spectral analysis of *1* indicated 84.9% d_2. Double bond location by ozonolysis showed no bond migration. Overall yield of *1* from the mixed *Vernonia* esters was approximately 60%.

Triglyceride of *cis*-12-Octadecenoic-9,10,15,15,16,16-d_6 acid (21). Into a 200-ml round-bottomed flask equipped with a magnetic stirrer and a N_2 inlet and outlet were placed dry glycerol (2.3 g, 0.025 mol), *cis*-12-octadecenoic-9,10,15,15,16,16-d_6 acid (24.33 g, 0.084 mol) and p-toluenesulfonic acid (0.2 g). The flask was heated with stirring in an oil bath at 120° while N_2 was used to sweep out the H_2O formed. At approximately 2-hr intervals fresh portions (0.1-0.2 g) of *p*-toluenesulfonic acid were added. The reaction was monitored by TLC using ethyl ether as developer and was cooled to room temperature when TLC showed only triglyceride and free fatty acid (approximately 12 to 22 hr, depending upon how often *p*-toluenesulfonic acid was added). A chromatographic column (22 mm I.D.) was packed with Florisil (60 g, Fisher, 100-200 mesh) slurried in isooctane, and a portion (8.38 g) of the triglyceride preparation was placed on the column. Three 100-ml fractions of isooctane yielded a total of 3.20 g of triglyceride, and four 100-ml portions of isooctane:ether = 90:10 yielded 4.07 g more. The column was stripped (0.5 g) with three 100-ml portions of ethyl ether. TLC using ethyl ether as developer showed the isooctane and isooctane:ether fractions to contain only triglycerides whereas the ether fraction contained many components. The remainder of the triglyceride preparation was treated in a similar manner and the total yield of triglyceride was 20.03 g or 88.8% of theory. The melting point of this triglyceride was 35-36°. The triglycerides of the other acids were prepared in a similar manner. The melting point of the triglyceride of *trans*-12-octadecenoic-15,15,16,16-d_4 acid was 51-52°, that of the triglyceride of *cis*-12-octadecenoic-15,15,16,16-d_4 acid was 27.5-28°, and that of the triglyceride of *trans*-12-octadecenoic-9,10-d_2 acid was 50-51°.

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