Analysis of Hydroxylated Fatty Acids from Plant Oils

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ABSTRACT: A novel process has been described recently for the preparation of hydroxylated fatty acids (HOFA) and HOFA methyl esters from plant oils. HOFA methyl esters prepared from conventional and alternative plant oils were characterized by various chromatographic methods (thin-layer chromatography, high-performance liquid chromatography, and gas chromatography) and gas chromatography–mass spectrometry as well as ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. HOFA methyl esters obtained from *Euphorbia lathyris* seed oil, low-erucic acid rapeseed oil, and sunflower oil contain as major constituents methyl *threo*-9,10-dihydroxy octadecanoate (derived from oleic acid) and methyl dihydroxy tetrahydrofuran octadecanoates, e.g., methyl 9,12-dihydroxy-10,13-epoxy octadecanoates and methyl 10,13-dihydroxy-9,12-epoxy octadecanoates (derived from linoleic acid). Other constituents detected in the products include methyl esters of saturated fatty acids (not epoxidized/derivatized) and traces of methyl esters of epoxy fatty acids (not hydrolyzed). The products that contain high levels of monomeric HOFA may find wide application in a variety of technical products.
JAOCS 72, 361–368 (1995).

KEY WORDS: Hydroxylated fatty acids, dihydroxy tetrahydrofuran octadecanoic acids, methyl 9,12-dihydroxy-10,13-epoxy octadecanoates, methyl 10,13-dihydroxy-9,12-epoxy octadecanoates, *threo*-9,10-dihydroxy octadecanoic acid (*threo*-9,10-dihydroxystearic acid).

Monohydroxy fatty acids occur in appreciable proportions in the seed oils of a few higher plants, for example, *Ricinus communis*, *Lesquerella fendleri,* and *Wrightia* spp. (1–3). Among the oils from these plants, only castor oil is used so far commercially in large amounts, and its major constituent, ricinoleic acid, i.e., 12-hydroxy-*cis*-9-octadecenoic acid, is the only hydroxy fatty acid that is used industrially for the preparation of a wide variety of technical products, such as sebacic acid, undecylenic acid, polyols for polyurethanes, detergents and lubricants (4).

Hydroxy fatty acids with more than one hydroxy group can be obtained by chemical functionalization of vegetable oils, such as rapeseed or soybean oil or fatty acids derived therefrom (5); such products are of great current interest in view of their possible use as starting materials for the production of plastics, e.g., polyurethanes and polyesters. A novel process for the preparation of hydroxylated fatty acids (HOFA) that offers economical and environmental advantages is described in an accompanying paper, which involves epoxidation of unsaturated fatty acids/acyl moieties of plant oils followed by catalytic opening of the oxirane ring in the presence of water (6). The present communication deals with the analysis of technical HOFA methyl esters, which were prepared by this procedure (6).

MATERIALS AND METHODS

Chemicals. High-performance liquid chromatography (HPLC)-grade solvents (acetonitrile, acetone) were purchased from E. Merck (Darmstadt, Germany). Hydroxy fatty acid standards were products of Sigma Chemie (Deisenhofen, Germany). Methyl esters of hydroxy fatty acid standards were prepared by using diazomethane (7). Trimethylsilyl (TMSi) ether derivatives of HOFA methyl esters were prepared by using *N,O-bis*(TMSi)-acetamide reagent (Macherey-Nagel, Düren, Germany) (8).

HOFA methyl esters derived from zero-zero-rapeseed oil, conventional sunflower oil as well as the seed oil of *Euphorbia lathyris* were technical products from Harburger Fettchemie Brinckman & Mergell (HOBUM) (Hamburg, Germany).

Analytical thin-layer chromatography (TLC). Samples of HOFA methyl esters derived from various plant oils were separated on layers of Silica Gel H (E. Merck) or Silica Gel H containing 5% boric acid (w/w) with hexane/diethyl ether (1:4, vol/vol). All lipid fractions on TLC plates were detected by charring after spraying with chromic–sulfuric acid (sulfuric acid/water/5% $K_2Cr_2O_7$, 10:10:1, vol/vol/wt) and heating at 200°C.

Preparative TLC of HOFA methyl esters. To determine the composition of HOFA methyl esters, samples of around 0.1 g derived from various plant oils were solubilized in dichloromethane/methanol (4:1, vol/vol), applied to five Silica gel H plates (layer thickness 0.5 mm) and developed with

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hexane/diethyl ether (1:4, vol/vol). Five lipid fractions (Fig. 1: fractions 1, R_f = 0.63–1.0; 2, R_f = 0.52–0.63; 3, R_f = 0.43–0.52; 4, R_f = 0.3–0.43; and 5, R_f = 0–0.3) were recovered from silica gel by repeated extraction with dichloromethane/methanol/water (65:35:1, vol/vol/vol). The various lipid extracts were concentrated, dried *in vacuo* at 50°C for several hours and weighed. Recovery was within 85–95%.

Gas chromatography (GC) of TMSi–ether derivatives of HOFA methyl esters. GC was carried out with a Hewlett-Packard HP-5890 Series II gas chromatograph (Palo Alto, CA). The TMSi–ether derivatives of HOFA methyl esters were separated on a 0.52 µm HP-1 fused-silica column (Hewlett-Packard), 25 m × 0.32 mm i.d., with helium (5.2 mL/min) as carrier gas and the following temperature program: 4 min at 150°C initially, followed by linear programming from 150 to 250°C at 10°C/min; finally, the temperature was held at 250°C for 16 min. The split ratio was 1:30; the temperature of the injector and thermal conductivity detector (TCD) was 270°C.

GC–mass spectrometry (GC–MS) of TMSi–ether derivatives of HOFA methyl esters. GC–MS of the TMSi–ethers was performed at electron impact (EI) mode (70 eV) and chemical ionization (CI) mode (methane as reagent gas) on a Hewlett-Packard instrument Model 5890 Series II/5989 A. The GC was equipped with a Permabond OV-1 fused-silica capillary column (25 m × 0,32 mm i.d.; 0.23 µm film thickness). The carrier gas was helium at a flow rate of 1.5 mL/min. The column temperature was initially kept at 200°C for 5 min, then programmed to 300°C at 4°C/min. The final temperature was held for 15 min. Other operating conditions were splitless injector temperature of 300°C, interface temperature of 280°C, and ion source temperature of 200°C.

HPLC of HOFA methyl esters. A Merck-Hitachi L-6200 pump equipped with an ACS 750/4 mass detector was used in combination with a Merck-Hitachi D-2000 ChromatoIntegrator for monitoring HPLC separations and measuring peak areas.

HOFA methyl esters were analyzed by reversed-phase HPLC on two LiChrospher 100 RP-18 (5 µm) columns (E. Merck), 125 × 4 mm each, in series with acetonitrile/water (9:1, vol/vol) isocratically at 0.6 mL/min.

Nuclear magnetic resonance (NMR) spectroscopy of HOFA methyl esters. ^1H and ^{13}C NMR spectra, ^{13}C APT (attached proton test) spectra, as well as two-dimensional $^1H/^1H$ homocorrelated NMR spectra and $^1H/^{13}C$ heterocorrelated NMR spectra were obtained on a Varian Gemini 200 spectrometer (Palo Alto, CA) in deuterochloroform with tetramethylsilane as internal standard.

RESULTS AND DISCUSSION

GC of technical HOFA methyl esters after conversion to TMSi–ether derivatives revealed the presence of 16:0 and 18:0 methyl esters together with methyl *threo*-9,10-dihydroxy octadecanoate (Fig. 2). Peak D consists of methyl dihydroxy

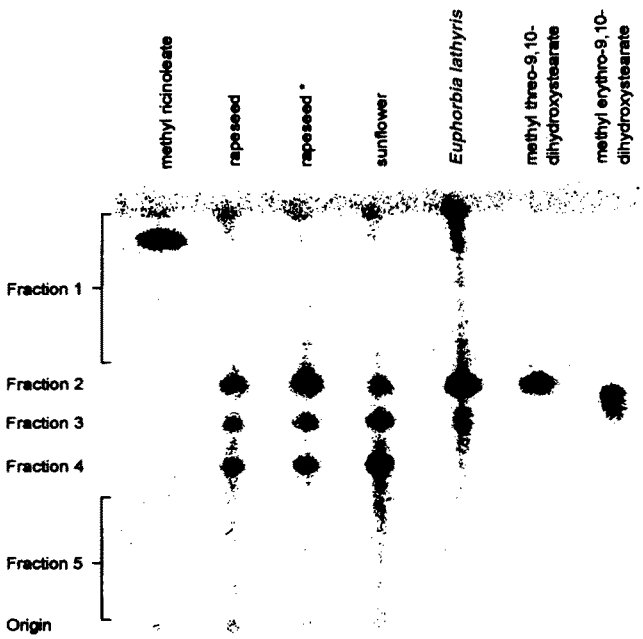


FIG. 1. Thin-layer chromatogram of technical hydroxylated fatty acid methyl esters derived from plant oils (*rapeseed = after additional saponification). Silica Gel H, hexane/diethyl ether, 1:4, vol/vol.

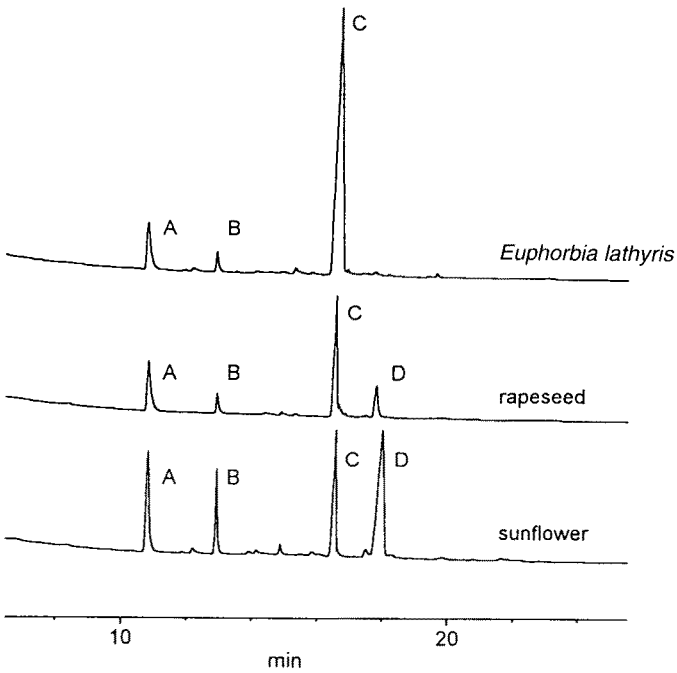


FIG. 2. Gas chromatography separation of the components of various technical hydroxylated fatty acid methyl esters prepared from the seed oil of *Euphorbia lathyris,* rapeseed oil, and sunflower oil after derivatization with *N,O-bis*(trimethylsilyl)-acetamide. A: 16:0, relative retention time (RRT) 1.00; B: 18:0, RRT 1.19 ; C: trimethylsilyl (TMSi)–ether derivative of methyl *threo*-9,10-dihydroxy octadecanoate, RRT 1.53; D: TMSi–ether derivatives of isomeric methyl dihydroxy tetrahydrofuran octadecanoates, RRT 1.66.

tetrahydrofuran (THF) octadecanoates (Fig. 2) as evident from GC–MS and NMR data given later.

The technical HOFA methyl esters from various plant oils were separated by TLC on layers of silica gel into five distinct lipid fractions according to their polarity (Fig. 2). TLC fraction 1 (Fig. 1) contained saturated fatty acid methyl esters as well as small proportions of epoxy C_{18} fatty acid methyl esters, judging from the GC–MS analysis, e.g., methyl 9,10-epoxy octadecanoate (*m/z* 312, M^+; 294, $M^+ - H_2O$), as well as methyl 9,10-epoxy-12-octadecenoate and methyl 12,13-epoxy-9-octadecenoate (*m/z* 310, M^+; 292, $M^+ - H_2O$). Fraction 1 also contained less polar estolides that were identified and quantitated by gel permeation chromatography (9).

Fraction 2 was identified as methyl *threo*-9,10-dihydroxy octadecanoate by comparison to standards with silica gel TLC (Fig. 1), boric acid–silica gel TLC, GC of the TMSi–ether derivative and reverse-phase HPLC. This identification of fraction 2 was confirmed by GC–MS of the TMSi–ether derivative and agreed with the mass spectrum of the authentic standard.

The TMSi–ether derivatives of both fractions 3 and 4 (Fig. 1) are not separated by GC (Peak D of Fig. 2). By HPLC, the underivatized fractions 3 and 4 yielded peaks that eluted at relative retention times, with reference to methyl *threo*-9,10-dihydroxyoctadecanoate, of 0.62 and 0.50, respectively (Fig. 3).

GC–MS data (Fig. 4) showed identical fragmentation for the TMSi–ether derivatives of both fractions 3 and 4, which were tentatively identified as methyl esters of dihydroxy THF octadecanoic acids, e.g., methyl 9,12-dihydroxy-10,13-epoxy octadecanoates and methyl 10,13-dihydroxy-9,12-epoxy octadecanoates (Scheme 1). Because no molecular ions were found in the EI–mass spectra of the TMSi–ether derivatives (Fig. 4) of both fractions 3 and 4 (Fig. 1), it was necessary to record CI mass spectra (Fig. 4, insert) to be able to determine the molecular weight (MW). The spectra of both fractions show a $(M + 1)^+$ peak at *m/z* 489, accompanied by small adduct ions at *m/z* 517 and 529, which are characteristic for CI–ionization with methane. The main fragments with *m/z* 399 $[(M^+ + 1) - (CH_3)_3SiOH]$ and *m/z* 302 $[(M^+ + 1) - 2(CH_3)_3SiOH]$ result from cleavage of one and two TMSi–OH moieties (*m/z* 90), respectively, indicating the presence of two hydroxy groups in the molecules.

The distinct signals at *m/z* 259 and 173 in the EI–mass spectra (Fig. 4) result from cleavages that are explained in the following MS fragmentation scheme of isomers of fraction 4 (Scheme 2).

The corresponding ions at *m/z* 229 and 315 appear as *m/z* 155 $[229 - (CH_3)_3SiH]$ and *m/z* 225 $[315 - (CH_3)_3SiOH]$, respectively (Scheme 2). These mass spectral data clearly indicate that both fractions 3 and 4 (Fig. 1) consist of mixtures of positional isomers of methyl dihydroxy THF octadecanoates. From these data, the MW of the underivatized substances are calculated as follows:

$$MW = \{(M + 1)^+ - 1)\} - \{2 \times (MW_{TMSiOH} - MW_{OH})\} + 2H \quad [1]$$

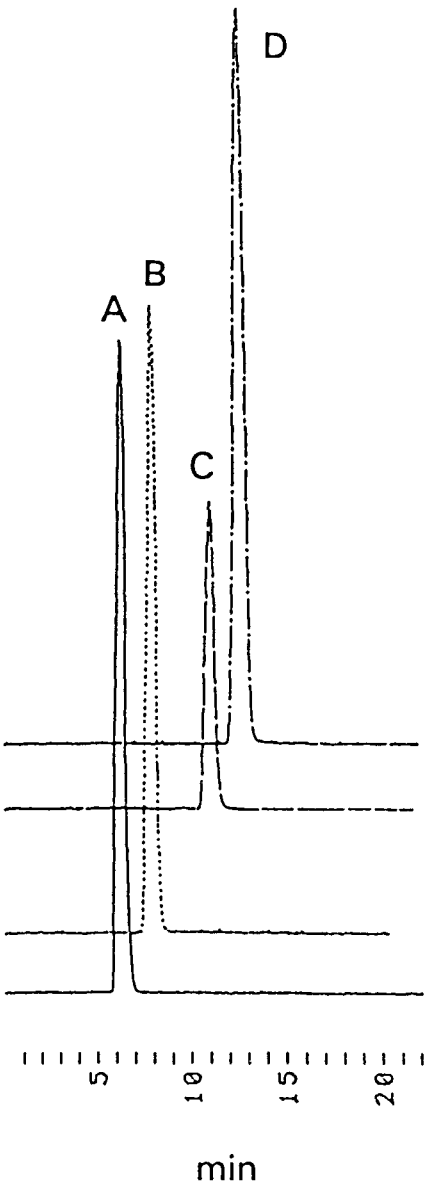
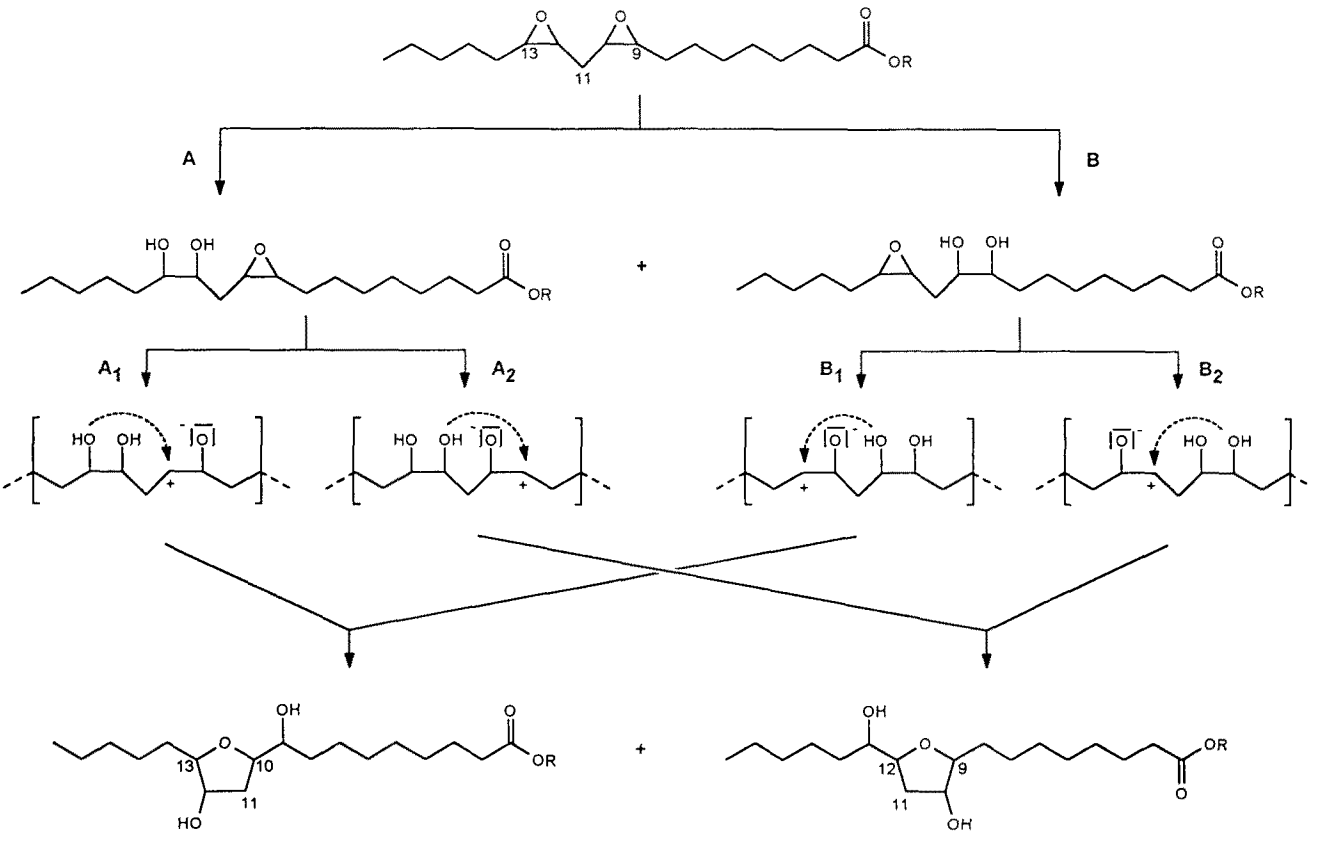


FIG. 3. High-performance liquid chromatography of fractions 3 and 4 (Fig. 1) of technical hydroxylated fatty acid methyl esters derived from plant oils. A: methyl 9,12-dihydroxy-10,13-epoxy octadecanoates and methyl 10,13-dihydroxy-9,12-epoxy octadecanoates (*trans* conformation; fraction 4, *cf.* Fig. 1) RRT 0.50; B: methyl 9,12-dihydroxy-10,13-epoxy octadecanoates and methyl 10,13-dihydroxy-9,12-epoxy octadecanoates (*cis* conformation; fraction 3, *cf.* Fig. 1) RRT 0.62; C: methyl *erythro*-9,10-dihydroxy-octadecanoate, standard, RRT 0.87; D: methyl *threo*-9,10-dihydroxy-octadecanoate, standard, RRT 1.00. See Figure 2 for abbreviation.

where $(M + 1)^+$ ion is derived from mass spectra of the silylated methyl dihydroxy THF octadecanoates, MW_{TMSiOH} is the MW of a TMSiOH fragment, and MW_{OH} the MW of a hydroxy group; the 2H are added to account for the loss of hydrogen during cleavage of two TMSiOH molecules. Consequently, the MW of the methyl dihydroxy THF octadecanoates are calculated as follows:

$$MW = \{489 - 1\} - \{2 \times (90 - 17)\} + 2 = 344 \quad [2]$$



SCHEME 1

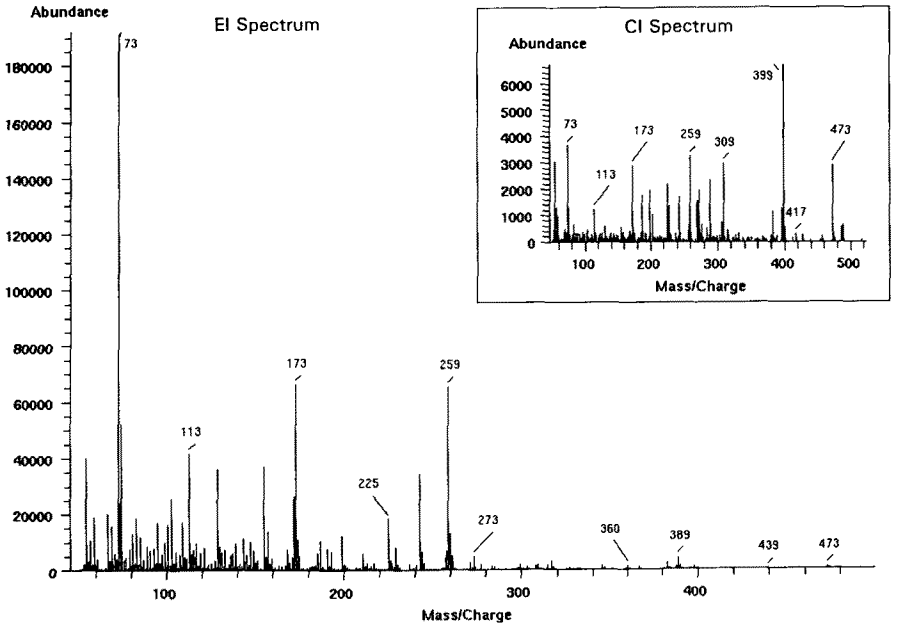
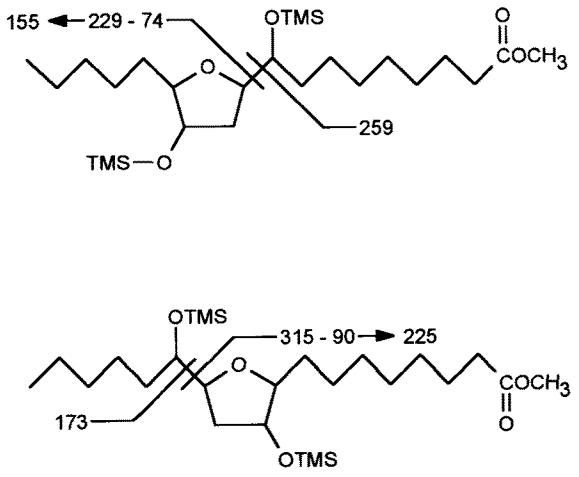


FIG. 4. Gas chromatography (GC)/electron impact (EI)–mass spectrometry of TMSi–ether derivative of fraction 4 (*cf.* Fig. 1) from technical hydroxylated fatty acid methyl esters of sunflower oil. The main GC-peak of both fractions 3 and 4 showed identical fragmentation as given in the fragmentation scheme. The assignments for the individual fragments are given in the text. The insert shows the corresponding chemical ionization (CI) mass spectrum. See Figure 2 for other abbreviation.



SCHEME 2

In both fractions 3 and 4 (Fig. 1) ^{1}H and ^{13}C NMR spectra as well as ^{13}C APT spectra reveal the presence of methyl ester groups (Tables 1–4). ^{13}C and ^{13}C APT NMR spectra show four signals between 60 and 80 ppm, which are assigned to four carbon atoms bound to oxygen (Tables 3 and 4). Most probably, two of these carbon atoms are substituted by hydroxy groups (*cf.* mass spectra, Fig. 4); two further C-atoms may be connected by an ether bridge (see below). The ^{13}C signal at 174.4 ppm is assigned to C-1, i.e., the >C=O group. From these observations, it can be concluded that the substances of both fractions 3 and 4 contain five oxygen atoms.

Assuming that the carbon chain of the C_{18}-methyl esters remained intact during epoxidation and oxirane ring opening, the sum formula of the substances of fractions 3 and 4 (Fig. 2) would be $C_{19}H_xO_5 = 344$, thus yielding 36 H atoms.

In mass spectra, as well as ^{1}H and ^{13}C NMR spectra of the fractions 3 and 4 (Fig. 1), there is no indication of the occurrence of C=C double bonds. However, the sum formula $C_{19}H_{36}O_5$ indicates two double-bond equivalents: the one is explained by the >C=O bond of the ester group, the other by a 1,4-epoxy (THF) ring.

Two-dimensional $^{1}H/^{1}H$ homocorrelated COSY spectra and $^{1}H/^{13}C$ heterocorrelated HETCOR spectra of fractions 3 and 4 (Fig. 1) were recorded to confirm the assignment of the various signals. It is obvious from the HETCOR spectra that both fractions 3 and 4 show only one ^{13}C signal at 38.85 and 38.04 ppm, respectively, which is correlated with two separate signals of two protons in the ^{1}H spectra of fraction 3 (1.83 and 2.37 ppm) and fraction 4 (1.86 and 2.01 ppm). This pattern of signals is typical of rigid $-CH_2-$ groups, e.g., those located in a ring system, and it is therefore assigned to the CH_2-11 of a THF ring (Tables 1 and 2) which is known to be formed from methyl hydroxy–epoxy octadecanoates (10–13) and other long-chain hydroxy–epoxy carboxylic acids (14) by treatment with acid or microsomal enzyme preparations (15,16) (Scheme 1). It is this particular C-11 methylene group that plays a key role for the assignment of protons of the THF ring.

The COSY spectra of fractions 3 and 4 (Fig. 1) show that the two protons of CH_2-11 couple with a high geminal coupling constant of 14.0 Hz (fraction 3) and 13.4 Hz (fraction 4). In addition, from the coupling of the two methylene protons at C-11 with the protons at 3.96 ppm (fraction 3) and 4.02 ppm (fraction 4), respectively, the assignment of these

TABLE 1
^{1}H NMR Assignment in Cisoid Isomeric Methyl 9,12-Dihydroxy-10,13-epoxy Octadecanoates (A) and Methyl 10,13-Dihydroxy-9,12-epoxy Octadecanoates (B) Derived from Sunflower Oil[a]

δ (ppm)	Number of protons	Assignment of ^{1}H NMR signals to the positional isomers	
		A	B
0.88	*t*, 3H	CH_3-18	CH_3-18
1.35	*m*, 14H	7 $(-CH_2-)_x$	7 $(-CH_2-)_x$
1.6	*m*, 6H	CH_2-3, CH_2-8 and CH_2-14	CH_2-3, CH_2-8 and CH_2-14
1.67	*s*, 1H	OH	OH
1.83	*dd*, 1H	CH-11$_\beta$ ($J_{H\text{-}11\alpha/H\text{-}11\beta}$ 14.0 Hz; $J_{H\text{-}10/H\text{-}11\beta}$ 3.4 Hz)	CH-11$_\beta$ ($J_{H\text{-}11\alpha/H\text{-}11\beta}$ 14.0 Hz; $J_{H\text{-}10/H\text{-}11\beta}$ 3.4 Hz)
2.3	*t*, 2H	CH_2-2 ($J_{H\text{-}2/H\text{-}3}$ 7.5 Hz)	CH_2-2 ($J_{H\text{-}2/H\text{-}3}$ 7.5 Hz)
2.37	*ddd*, 1H	CH-11$_\alpha$ ($J_{H\text{-}11\alpha/H\text{-}12}$ 5.5 Hz; $J_{H\text{-}11\alpha/H\text{-}10}$ 9.8 Hz; $J_{H\text{-}11a/H\text{-}11\beta}$ 14.0 Hz)	CH-11$_\alpha$ ($J_{H\text{-}11\alpha/H\text{-}10}$ 5.5 Hz; $J_{H\text{-}11\alpha/H\text{-}12}$ 9.8 Hz; $J_{H\text{-}11\alpha/H\text{-}11\beta}$ 14.0 Hz)
3.0–3.4	*s* (broad); 1H	OH	OH
3.48	*t* (broad); 1H	CH-9	CH-13
3.62	*m*, 1H	CH-13	CH-9
3.66	*s*, 3H	CH_3-O	CH_3-O
3.96	*m*, 1H	CH-10	CH-12
4.05	*m*, 1H	CH-12	CH-10

[a]*d* = Doublet; *dd* = doublet of doublets; *m* = multiplet; *s* = singlet; *t* = triplet; J = coupling constant (Hz); NMR, nuclear magnetic resonance.

TABLE 2
^{1}H NMR Assignment in Transoid Isomeric Methyl 9,12-Dihydroxy-10,13-epoxy Octadecanoates (A) and Methyl 10,13-Dihydroxy-9,12-epoxy Octadecanoates (B) Derived from Sunflower Oil[a]

δ (ppm)	Number of protons	Assignment of ^{1}H NMR signals to the positional isomers	
		A	B
0.88	*t*, 3H	CH_3-18	CH_3-18
1.3	*m*, 16H	8 $(-CH_2-)_x$	8 $(-CH_2-)_x$
1.6	*m*, 4H	CH_2-3, CH_2-8	CH_2-3, CH_2-14
1.67	*s*, 1H	C-12-OH	C-10-OH
1.86	*ddd(d)*, 1H	CH-11$_\beta$ ($J_{H\text{-}11\alpha/H\text{-}11\beta}$ 13.4 Hz; $J_{H\text{-}10/H\text{-}11\beta}$ 9.0 Hz; $J_{H\text{-}11\beta/H\text{-}12}$ 3.4 Hz)	CH-11$_\beta$ ($J_{H\text{-}11\alpha/H\text{-}11\beta}$ 13.4 Hz; $J_{H\text{-}12/H\text{-}11\beta}$ 9.0 Hz; $J_{H\text{-}11\beta/H\text{-}10}$ 3.4 Hz)
2.01	*dd*, 1H	CH-11$_\alpha$ ($J_{H\text{-}10/H\text{-}11\alpha}$ 6.8 Hz; $J_{H\text{-}11\alpha/H\text{-}11\beta}$ 13.4 Hz)	CH-11$_\alpha$ ($J_{H\text{-}11\alpha/H\text{-}12}$ 6.8 Hz; $J_{H\text{-}11\alpha/H\text{-}11\beta}$ 13.4 Hz)
2.3	*t*, 2H	CH_2-2 ($J_{H\text{-}2/H\text{-}3}$ 7.7 Hz)	CH_2-2 ($J_{H\text{-}2/H\text{-}3}$ 7.7 Hz)
2.37	*s*, 1H	C-9-OH	C-13-OH
3.38	*m*, 1H	CH-9	CH-13
3.66	*s*, 3H	CH_3-O	CH_3-O
3.75	*m*, 1H	CH-13	CH-9
4.02	*dt*, 1H	CH-10 ($J_{H\text{-}9/H\text{-}10}$ 6.7 Hz; $J_{H\text{-}10/H\text{-}11\beta}$ 9.0 Hz; $J_{H\text{-}10/H\text{-}11\alpha}$ 6.7 Hz)	CH-12 ($J_{H\text{-}12/H\text{-}13}$ 6.7 Hz; $J_{H\text{-}11\beta/H\text{-}12}$ 9.0 Hz; $J_{H\text{-}11\alpha/H\text{-}12}$ 6.7 Hz)
4.22	*m*, 1H	CH-12	CH-10

[a]See Table 1 for abbreviations.

signals to CH-10{12} (in brackets: assignment to the corresponding positional isomer; *cf.* Scheme 1) is evident. In addition, the proton at C-10{12} couples with the CH-9{13} proton. The COSY spectra of fractions 3 and 4 also show the coupling of CH-12{10} with CH-13{9} at 3.62 and 3.75, respectively (Tables 1 and 2). Relatively weak crosspeaks were found, however, for various other couplings, e.g., the methylene protons at C-11 with the CH-12{10} proton, as well as of the CH-13{9} proton with the two neighboring CH_2-14{8} protons, and of the CH-9{13} proton with the neighboring O–H proton or with the neighboring CH_2-8{14} protons at about 1.4 ppm. These observations are the basis for the

TABLE 3
^{13}C NMR Assignment in Cisoid Isomeric Methyl 9,12-Dihydroxy-10,13-epoxy Octadecanoates (A) and Methyl 10,13-Dihydroxy-9,12-epoxy Octadecanoates (B) Derived from Sunflower Oil

δ (ppm)	Assignment[a] of ^{13}C NMR signals to the positional isomers	
	A	B
174.35	C-1	C-1
34.17	C-2	C-2
73.98 (74.05)[b]	C-9	C-13
79.22	C-10	C-12
38.85	C-11	C-11
71.70	C-12	C-10
84.41 (84.36)[b]	C-13	C-9
22.67	C-17	C-17
14.09	C-18	C-18
51.50	CH_3-O	CH_3-O

[a]The following signals belong to C-atoms of the radyl moieties of the two positional isomers, which cannot be assigned unambiguously: 24.97, 25.01, 25.75, 25.97, 26.00, 26.21, 28.85, 29.12, 29.18, 29.21, 29.38, 29.67, 31.84, 32.12, 34.42, 34.45. Some of these signals are partially superimposed.
[b]Assignments of these ^{13}C nuclear magnetic resonance (NMR) signals are interchangeable between the two carbon atoms marked.

TABLE 4
^{13}C NMR Assignment in Transoid Isomeric Methyl 9,12-Dihydroxy-10,13-epoxy Octadecanoates (A) and Methyl 10,13-Dihydroxy-9,12-epoxy Octadecanoates (B) Derived from Sunflower Oil

δ (ppm)	Assignment[a] of ^{13}C NMR signals to the positional isomers	
	A	B
174.37	C-1	C-1
34.15 (34.17)[b]	C-2	C-2
74.14 (74.19)[b]	C-9	C-13
80.27 (80.30)[b]	C-10	C-12
38.04	C-11	C-11
73.54	C-12	C-10
82.54 (82.60)[b]	C-13	C-9
24.96 (25.00)[b]	C-17	C-17
14.09 (14.12)[b]	C-18	C-18
51.51	CH_3-O	CH_3-O

[a]The following signals belong to C-atoms of the radyl moieties of the two positional isomers which cannot be assigned unambiguously: 24.96, 25.00, 25.36, 25.59, 26.07, 26.26, 28.93, 29.08, 29.15, 29.24, 29.53, 29.60, 32.00, 32.07, 33.24, 33.27, 34.15, 34.17. Some of these signals are partially superimposed.
[b]Assignments of these ^{13}C nuclear magnetic resonance (NMR) signals are interchangeable between the two carbon atoms marked.

assignments of the various protons as shown in Tables 1 and 2.

The coupling constants of the methylene protons at C-11 with their various neighboring protons are helpful in giving some ideas about the position and stereochemical arrangement of the substituents at the THF ring, e.g., hydroxy groups and radyl moieties. Although both fractions consist of at least two isomers in nearly equal amounts, as is evident from the number and intensities of signals in the ^{13}C-spectra, five coupling constants are observed for the signals of the two CH_2-11 protons. If both the hydroxy group at C-12{10} and its neighboring radyl moiety at C-13{9} are located above the plane of the THF ring, then we indicate the proton at C-11, which is also located above the plane of the THF ring, as H-11_α, while the proton that is below the plane is indicated as H-11_β. The H-11_β proton of fraction 4 at 1.86 ppm shows three coupling constants, i.e., the geminal H-11_α/H-11_β coupling as well as $J_{H\text{-}11\beta/H\text{-}10}$ and $J_{H\text{-}11\beta/H\text{-}12}$, whereas the H-$11_\alpha$ proton at 2.01 ppm shows only two coupling constants, i.e., $J_{H\text{-}11\alpha/H\text{-}11\beta}$ and $J_{H\text{-}11\alpha/H\text{-}10}$ (Table 2). No coupling $J_{H\text{-}11\alpha/H\text{-}12}$ has been observed in fraction 4.

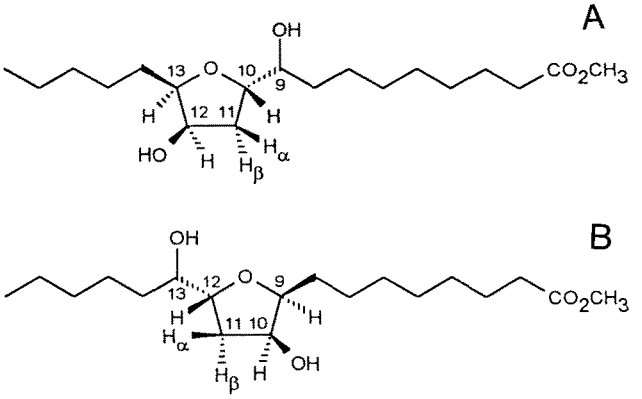
The CH-11_α proton (2.37 ppm) of fraction 3 shows three coupling constants (Table 1), whereas the proton CH-11_β at 1.83 ppm has only two coupling constants. In contrast to fraction 4, no coupling $J_{H\text{-}11\beta/H\text{-}12}$ was found in fraction 3. This coupling cannot be observed, if the coupling constant is about zero, which is the case when the dihydro angle between two neighboring protons is nearly 90°.

It is difficult to determine the relative configuration of a five-membered ring through coupling constants alone, and it is impossible to determine the absolute configuration. X-Ray analysis can solve this problem. This was done in 1980 on a similar alkenoic compound, i.e., 6,9-epoxy nonadec-18-ene-7,10-diol (17). This compound was found to have the configuration 6*S*,7*S*,9*R*,10*R*, showing that the arrangement of the hydroxy group at the THF ring and its neighboring radyl moiety is cisoid, while the arrangement of the two radyl moieties is transoid.

Because the ^{1}H and ^{13}C NMR data of the THF ring in fraction 4 have the same values as found by Warren *et al.* (17), it is likely that the relative configuration of fraction 4 is transoid for the two bulky radyl moieties. From all these observations, it is concluded that fraction 4 (Fig. 1) consists of positional isomers and stereoisomers of the structure shown in Scheme 3 (15,16).

Conversely, it can be concluded that fraction 3 (Fig. 1) contains the positional isomers of methyl dihydroxy THF octadecanoates with cisoid arrangement of the two long-chain radyl moieties as well as a cisoid arrangement of the hydroxy group of the THF ring and the neighboring radyl substituent. Such compounds were previously isolated by Hammock and co-workers (15,16), who established the sterical arrangement of the radyl moieties by various spectrophysical methods. Our data are in good agreement with these values.

Taking all these findings into account, we identify the fractions 3 and 4 (Fig. 1) as positional and stereoisomers of



SCHEME 3

methyl dihydroxy THF octadecanoates, e.g., methyl 9,12-dihydroxy-10,13-epoxy octadecanoates and methyl 10,13-dihydroxy-9,12-epoxy octadecanoates. All chromatographic and spectroscopic data agree well with those given in the literature (15,16). Formation of a symmetrically substituted dihydroxy tetrahydropyran ring, which is also thinkable (18), can be ruled out from the relatively complex pattern of NMR signals of both fractions 3 and 4.

In this context, it has to be kept in mind that the compounds investigated were products of technical organic synthesis, so that the formation of racemic mixtures of compounds is likely.

Fraction 5 (Fig. 1) was found to be predominantly constituted of polar estolides of HOFA as evidenced by gel permeation chromatography (9) and additional saponification (Table 5).

Compositions of the individual fractions, differing in polarity, in the technical HOFA methyl ester products, as determined by preparative TLC, are summarized in Table 5. This distribution of reaction products according to their polarity broadly reflects—as to be expected (Fig. 1)—the contents of oleic acid and linoleic acid, respectively, in the starting materials. For example, *E. lathyris* seed oil which contains *ca.* 80% of oleic acid in the starting material, yielded *ca.* 50% methyl *threo*-9,10-dihydroxy octadecanoate (fraction 2), whereas sunflower oil, which contains *ca.* 65% linoleic acid, yielded a total of *ca.* 40% methyl dihydroxy THF octadecanoates in fractions 3 and 4.

Under acidic conditions, the ring opening of the epoxy fatty acid methyl esters leads at first to oxonium ions, which react subsequently with water or nucleophilic groups, e.g., intra- and intermolecular hydroxy groups. As a consequence, a number of different reaction products are to be expected if a particular direction of the oxirane ring opening fails.

Under the conditions used for the preparation of HOFA (6), dihydroxy THF octadecanoic acids were obviously the preferred reaction products. Opening of the oxirane rings of 9,10-12,13-diepoxy octadecanoyl moieties and subsequent intramolecular nucleophilic attack obviously occur for both the 9,10- and 12,13-epoxy groups with almost equal proba-

TABLE 5
Composition of Methyl Esters of Hydroxylated Fatty Acids (HOFA) from Various Plant Oils

	TLC fractions[a]			
	(1)	(2)	(3 + 4)	(5)
HOFA methyl esters from	Nonpolar	Dihydroxy	Dihydroxy-THF (wt%)	Polar
Euphorbia lathyris oil	39	52	9[b]	—
Rapeseed oil	38	36	16	10
Rapeseed oil (after additional saponification)	34	44	17	5
Sunflower oil	31	17	34	18
Sunflower oil (after additional saponification)	22	22	41	15

[a]*cf.* Figure 1; TLC, thin-layer chromatography; THF, tetrahydrofuran.
[b]Including small proportions of fraction 5.

bility. This is evident from the ^{13}C spectra of fractions 3 and 4, which show various pairs of signals with almost equal intensity, indicating an equal distribution of the two positional isomers in each fraction. The comparison of yields of reaction products showed a distribution of fraction 3/fraction 4 ≈ 3:4, indicating a slight preference for fraction 4. Assuming that the THF ring formation is the thermodynamically preferred reaction, two series of intramolecular substitution products (A_1, A_2, B_1, and B_2) are possible, starting from the partly hydrolyzed intermediates A and B. As shown in Scheme 1, the intermediates A_1 and B_1 as well as A_2 and B_2 finally lead to the same series of positional isomers. In this context, it is of interest that thermal dimerization of linoleic acid at 265°C leads to both 2,5-disubstituted THF- and 2,6-disubstituted tetrahydropyran-derivatives (19).

Methyl 9,10-12,13-diepoxy octadecanoate, which may be a metabolite of linoleic acid, forms the same dihydroxy THF fatty acids—as they have been found in technical HOFA products—during *in vitro* incubation in the presence of cytosolic epoxide hydrolase (15,16). Various furan carboxylic acids ("F acids"), including THF-carboxylic acids, have been detected in blood and urine of humans and animals as well as in wool fat (13,20–22). Recently, similar dihydroxy THF hydrocarbons, including estolides formed therefrom, have been isolated from the brown alga *Notheia anomala* (17,23,24).

ACKNOWLEDGMENTS

We thank H. Becker, D. Kampmeier, I. Kiewitt, E. Klein, and H. Lutter for excellent technical assistance.

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[Received May 12, 1994; accepted September 1, 1994]