Autoxidative Dimerization of Methyl Linolenate and Its Monohydroperoxides, Hydroperoxy Epidioxides and Dihydroperoxides

W.E. Neff, E.N. Frankel* and **K. Fujimoto**[1]
Northern Regional Research Center, ARS/USDA, 1815 N. University St., Peoria, IL 61604

The structures of dimers and oligomers produced by autoxidation of methyl linolenate and its purified oxidation products were investigated to obtain a better understanding of the mechanism of oxidative deterioration of unsaturated lipids. The dimers were separated by gel permeation chromatography, characterized by molecular weight determinations before and after sodium borohydride reduction, and analyzed by ultraviolet, infrared, ^{1}H NMR and fast atom bombardment mass spectrometry. Autoxidation of methyl linolenate at 40 C to peroxide value of 1062 produced 6.8% dimers mainly derived from hydroperoxides, hydroperoxy epidioxides and dihydroperoxides. These dimers were 88% peroxide-linked (C-O-O-C) and 12% ether- (C-O-C) and/or carbon-linked (C-C). Autoxidation of methyl linolenate monohydroperoxides at 40 C produced dimers that were 72% peroxide- and 28% ether/carbon-linked. Thermal decomposition of linolenate hydroperoxides at 150 C gave dimers that were 100% ether/carbon-linked, and catalytic decomposition with ferric chloride-ascorbic acid at room temperature gave dimers with 43% peroxide and 57% ether/carbon linkages. Autoxidation of linolenate hydroperoxy epidioxides at 40 C produced dimers containing hydroperoxy epidioxides, dihydroperoxides and monohydroperoxides joined with peroxide and ether/carbon linkages. Under the same conditions, autoxidation of linolenate dihydroperoxides produced dimers containing dihydroperoxides and hydroperoxy epidioxides joined with peroxide and ether/carbon linkages. These dimers contribute to oxidative and flavor deterioration of polyunsaturated fats in the same way as the hydroperoxide precursors by further decomposition to produce volatile compounds.

Much progress has been made in the last decade to clarify the structures of the monomeric secondary oxidation products and volatile decomposition products of methyl linoleate and linolenate (1–9). However, not much structural information is yet available on the secondary oxidation products of high molecular weight, which appear to be important in linoleate (2) and linolenate (6).

Early studies have shown that unsaturated fats polymerize during autoxidation (10–14) and that the resulting oxidative dimers and polymers have an impact on the oxidative and flavor stability of vegetable oils (15,16). The thermal polymerization of oxidized methyl linolenate in the absence of air produced a complex mixture of oxygen-containing compounds, including 70% dimers; the rest consisted of trimers, oligomers and carbonyl and hydroxy compounds (12). These oxygenated compounds of high molecular weight can decompose to generate volatile aldehydes (17).

Although several structural studies have been published recently on the oxidative dimers of linoleate and its hydroperoxides, no attention has been given to the dimer compounds formed during autoxidation of methyl linolenate. Dimers containing peroxide linkages were identified during the initial stages of autoxidation of methyl linoleate at room temperature (18–20). The dimers isolated from the autoxidation of methyl linoleate hydroperoxides at room temperature were composed of unsaturated fatty ester units crosslinked through either peroxide or ether linkages and contained hydroperoxy, hydroxy and oxo groups (21).

Peroxy-linked dimers also were reported from the anaerobic reaction between linoleic acid hydroperoxides and the free radical initiator di-t-butyl peroxyoxalate at 38 C (3), and between the linoleate 13-hydroperoxide isomer and copper(II) palmitate (22). On the other hand, a carbon-linked dimer was identified from the copper-catalyzed decomposition of a linoleate 13-hydroperoxide concentrate (95%) (23). Oxidative decomposition of the linoleate dimers at room temperature produced hydroxy esters, hydroxy aldehydes, nonenal, aldehyde esters and oxygenated aldehyde esters (24).

In a previous study (6), the fractionation of autoxidized methyl linolenate by silicic acid column chromatography produced 3.7 to 5.9% of a polar fraction that was not identified but presumed to include higher molecular weight materials. In this paper, we characterized the high-molecular weight products from methyl linolenate autoxidized at 40 C. Dimers from pure methyl linolenate monohydroperoxides, cyclic peroxides (hydroperoxy epidioxides) and dihydroperoxides were characterized to elucidate the mechanism of oxidative polymerization. We also compared the dimers from methyl linolenate hydroperoxides decomposed thermally at 150 C and catalytically with ferric chloride and ascorbic acid. In another paper, we report the volatile thermal decomposition products formed from purified dimer fractions of autoxidized methyl linolenate and derived oxidation products (25).

EXPERIMENTAL PROCEDURES

Materials. Pure methyl linolenate (6) was autoxidized at 40 C with pure oxygen (2) to reach peroxide values ranging between 585 and 4,000 me/kg. A sample oxidized to a peroxide value of 1,000 was used to prepare pure monohydroperoxides, hydroperoxy epidioxides and dihydroperoxides by chromatographic procedures described previously (6). Purity of the oxidation products was checked by thin layer chromatography (TLC) with previously characterized oxidation materials as references (6).

Polymerization. Oxidative polymerization was carried out with 100 to 1,000 mg neat samples of pure

*To whom correspondence should be addressed.
[1]Visiting scientist from Tohoku University, Sendai, Japan.

methyl linolenate, monohydroperoxides, hydroperoxy epidioxides, or dihydroperoxides in a test tube by bubbling oxygen through them at 40 C. The reaction was stopped by freezing the products at −20 C when analysis by TLC (silica gel, solvent: diethyl ether/hexane, 1:1, v/v) indicated about 80–100% disappearance of the starting material. Thermal polymerization of methyl linolenate monohydroperoxides was done under oxygen at 150 C and catalytically with ferric chloride and ascorbic acid at room temperature by the procedures described previously (26). The conditions used for the catalytic decomposition were similar to those used previously for fluorescence formation in the presence of DNA (27).

Gel permeation chromatography (GPC). A high performance liquid chromatograph (DuPont Instruments, Wilmington, Delaware) was used with two glass columns 9 × 1,000 mm) connected in series and packed with Bio-Beads S-X2 (200–400 mesh, Bio-Rad Labs, Richmond, California). Methylene chloride was employed as mobile phase at a flow rate of 0.5 ml/min and pressure of 16 to 19 bars. Samples of 100 μl dissolved in methylene chloride were injected, and the separation was followed with refractive index (Waters Associates, Millford, Massachusetts) and ultraviolet (DuPont, at 265 nm) detectors. Average molecular weights (mw) of recovered fractions were determined by vapor pressure osmometry with benzene as solvent (28).

Analytical methods. TLC of purified dimer fractions was performed on silica gel plates with a solvent mixture of methanol:chloroform (5/95, v/v). Reversed phase TLC was done with C_{18} plates (Whatman, Clifton, New Jersey) and methanol as solvent. Peroxide-active spots were detected with a 10% KI solution. This reagent is known to reduce hydroperoxide groups readily but not epidioxide and peroxy bonds (3,29). Ultraviolet (UV), infrared (IR), ^{1}H NMR, and gas chromatography-mass spectrometric (GC-MS) analyses were carried out by the procedures described previously (6). Selected dimer fractions were also analyzed by fast atom bombardment mass spectrometry (FABMS) (30). Samples were run on a Nuclide 12-90-DF mass spectrometer with a xenon fast atom bombardment gun. The samples were placed in the 10 kV xenon atom beam on a probe with glycerol as the matrix.

Dimers were further characterized by analyzing the monomeric components formed after either $NaBH_4$ reduction or catalytic hydrogenation. Although $NaBH_4$ reduces hydroperoxide groups and cleaves peroxide bonds in linoleate dimers (3,18,35), epidioxide bonds are reported to be resistant to this reducing agent (31). Catalytic hydrogenation, on the other hand, reduces hydroperoxides, epidioxides and peroxy bonds in linoleate dimers (2,3,6,29,31). Dimer fractions were reduced by adding, in small portions, a 100-mg sample dissolved in a mixture of methanol and water (1/1, v/v) to a solution of 200 mg $NaBH_4$ in 0.1 N KOH, with stirring for two hr at room temp. The product was neutralized with acetic acid, the methanol evaporated, and the residue extracted with diethyl ether and dried over sodium sulfate. Dimer samples (5–10 mg) were also hydrogenated with platinum oxide catalyst in ethanol solution. The products reduced with $NaBH_4$ and hydrogenated were analyzed by TLC (silica gel, diethyl ether:hexane, 1:1, v/v) to check for completeness of reaction, and for molecular weight to determine the type of linkages involved in the dimers. Hydrogenated products were silylated with a commercial reagent, Sylon BTZ (Supelco, Inc., Bellfonte, Pennsylvania), and analyzed by capillary GC and GC-MS.

Capillary gas chromatography and GC-MS. A Hewlett-Packard instrument (Model 5700, Palo Alto, California) was used with a capillary column (DB-1, J & W Scientific Co., Rancho Cordova, California; 0.32 × 15,000 mm), isothermally at 250 C with a helium flow of 1.32 ml/min and a split ratio of 46:1. Peak areas were inte-

TABLE 1

Relative Weight-Percent Composition of Gel Permeation Chromatographic (GPC) Fractions from the Oxidative Decomposition of Methyl Linolenate and Purified Oxidation Products[a]

Oxidation samples	Decomposition conditions	GPC-1		GPC-2		GPC-3	
		wt%	mw	wt%	mw	wt%	mw
Autoxidized linolenate							
PV 585	40 C	3.2	840	96.7	380	0.1	—
PV 1062	40 C	6.8	808	92.7	375	0.5	187
PV 4002	40 C	54.6	825	44.4	367	1.0	—
Mono-hydroperoxides	40 C	75.3	726	24.6	377	0.1	202
Mono-hydroperoxides	150 C	49.3	834	50.1	410	0.6	195
	Fe-ascorbic[b]	53.9	625	45.6	277	0.5	185
Hydroperoxy epidioxides	40 C	90.0	930	9.5	427	0.5	212
Dihydroperoxides	40 C	45.0	710	55.0	363	1.0	199

[a]See Fig. 1. Abbreviations: PV, peroxide values; mw, molecular weight (by vapor pressure osmometry).
[b]Ferric chloride-ascorbic acid, room temperature (27).

grated by computer. Peak components were identified by matching with known standards of mono-, di-and trihydroxystearates, and by GC-MS (2,31).

RESULTS AND DISCUSSION

The products of oxidative polymerization of methyl linolenate were separated by GPC into three fractions (GPC-1, GPC-2, GPC-3) with mean molecular weight corresponding to dimer/oligomer, monomer, and low molecular weight components. A sample prepared from methyl linolenate autoxidized at 40 C to a peroxide value of 1062 me/kg produced 6.8% GPC-1 of average molecular weight 808, 92.7% GPC-2 of average molecular weight 375, and 0.5% GPC-3 of average molecular weight 187 (Fig. 1, Table 1). In samples oxidized to peroxide values of 585 and 4,002, the dimeric GPC-1 fractions varied from 3.2 to 54.6%, respectively (Table 1). The relative proportions of GPC-1 and GPC-2 fractions were directly related to the peroxide values of the autoxidized methyl linolenate (Table 1). Dimer and monomer components were UV- and peroxide-positive. The UV-active monomeric components eluting in the front portion of the GPC-2 peak apparently are due to secondary oxidation products, while the unoxidized linolenate eluted in the back portion of this peak. TLC examination of monomeric GPC-2 fractions with a diethyl ether:hexane solvent mixture (1/1, v/v) showed components with the same R_f as epoxy and keto esters, monohydroperoxides, hydroperoxy epidioxides, and dihydroperoxides, previously identified in autoxidized methyl linolenate (6). The low molecular weight components in the GPC-3 fraction correspond to aldehydes and esters (26).

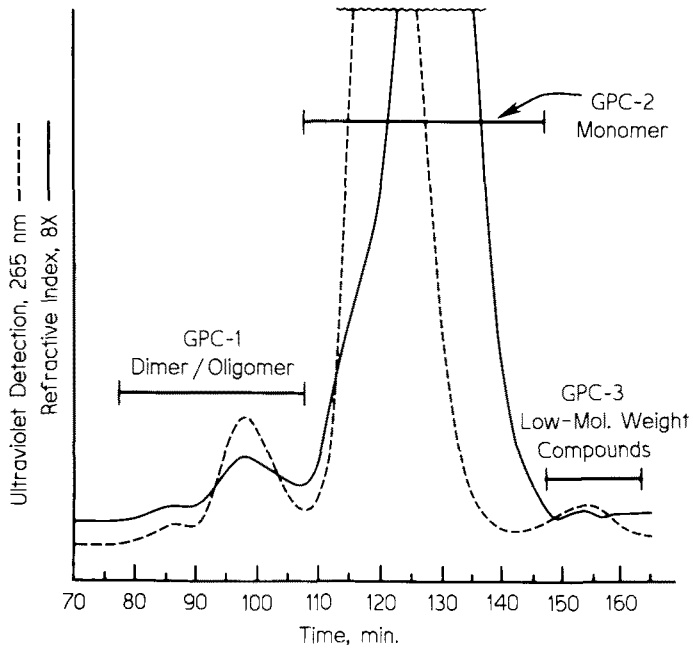

FIG. 1. Gel permeation chromatography of methyl linolenate autoxidized at 40 C to a peroxide value of 1062 me/kg. Solid line, refractive index detection; broken line, ultraviolet detection.

The composition of the monomer fractions from oxidized methyl linolenate suggested that the same components were involved in the dimeric products. Therefore, linolenate monohydroperoxides, hydroperoxy epidioxides and dihydroperoxides were oxidatively polymerized at 40 C under the same conditions as methyl linolenate. Fractionation by GPC produced 75% dimer (GPC-1) from monohydroperoxides, 90% from hydroperoxy epidioxides, and 45% from dihydroperoxides, with respective molecular weights of 726, 930 and 710 (Table 1). The GPC-1 fraction isolated from the thermal polymerization of linolenate monohydroperoxides at 150 C equalled 49% and had a molecular weight of 834, apparently due to the presence of oligomers and polymers. The corresponding fraction isolated from the catalytic polymerization of linolenate hydroperoxides in the presence of ferric chloride and ascorbic acid equalled 54% and was mainly dimeric with a molecular weight of 625. The monomeric GPC-2 fractions varied from 9.5 to 90% with molecular weights ranging from 277 to 410. The GPC-3 fractions were minor with all oxidation products (0.1-1.0%) and had mean molecular weights ranging from 187 to 212.

Dimers with peroxide linkages are known to cleave by either $NaBH_4$ reduction (18) or catalytic hydrogenation (3) to produce the monomeric components. Dimers with ether or carbon linkages, however, are resistant to cleavage under these conditions. To determine the nature of the linkages between monomeric units, the dimeric fractions were reduced with $NaBH_4$, and the resulting products were again fractionated by GPC. The amount of peroxide linkage in the dimers was calculated by determining the increase in the monomeric GPC-2 fraction observed after $NaBH_4$ reduction. The percentage of peroxide linkage in the dimeric GPC-1 fractions was 88.3% in methyl linolenate autoxidized to a peroxide value of 1,062, compared to 55.6% in methyl linoleate autoxidized to a peroxide value of 2,000 (Table 2). The calculated amount of peroxide linkage in the dimeric fraction of methyl linolenate hydroperoxides autoxidized at 40 C was 71.7%, compared to 51% in methyl linoleate hydroperoxides autoxidized under the same conditions (Table 2). In contrast, after thermal polymerization at 150 C, the dimeric fraction from methyl linolenate hydroperoxides contained no peroxide linkage, whereas after catalytic polymerization with ferric chloride at room temperature, the dimer fraction contained 43.2% peroxide linkages. After oxidative polymerization at 40 C, the proportions of peroxide linkages in the dimeric fractions were 71% in the hydroperoxy epidioxides and 82% in the dihydroperoxides (Table 2).

A previous study of methyl linoleate oxidized at 32 C to peroxide value of 16 showed that more than 90% of the dimer fraction was depolymerized with $NaBH_4$ and, therefore, joined by peroxide linkage (18). Another paper reported that in methyl linoleate hydroperoxides autoxidized at 30 C, two peroxide-linked dimers (30.5 and 17.1%) were formed, that were depolymerized with $SnCl_2$, and three ether-linked dimers (4.2, 5.5 and 17.0%) that were not depolymerized (21).

Dimers from autoxidized methyl linolenate. Several attempts were made to separate the dimeric components in GPC-1 fractions of autoxidized methyl lino-

TABLE 2

Proportion of Peroxide Linkage (C-OO-C) in Dimer Fractions by Gel Permeation Chromatographic (GPC) Analysis after Sodium Borohydride Reduction (Weight Percent)[a]

Oxidation samples	Decomposition conditions	GPC-1[b]		GPC-2	GPC-3
		C-OO-C	C-O-C/C-C		
Autoxidized[c]					
Linolenate	40 C	8.3 (88.3)	1.1 (11.7)	89.8	0.5
Linoleate	40 C	6.0 (55.6)	4.8 (44.4)	87.2	0.5
Linolenate					
Hydroperoxides	40 C	54.0 (71.7)	21.3 (28.3)	24.6	0.1
	150 C	0.0 (0.0)	49.3 (100)	50.1	0.6
	Fe-ascorbic[d]	23.3 (43.2)	30.6 (56.7)	45.6	0.5
Linoleate-OOH	40 C	21.9 (51.0)	21.0 (49.0)	24.6	0.1
Linolenate					
OOH Epidioxides	40 C	63.9 (71.0)	26.1 (29.0)	9.5	0.5
Dihydroperoxides	40 C	36.9 (82.0)	8.1 (18.0)	54.8	0.2

[a]Fig. 1. Abbreviations: C-OO-C, peroxide linkage; C-O-C, ether linkage; C-C, carbon linkage; OOH, hydroperoxide/hydroperoxy.

[b]Proportion of peroxide-linked dimers determined by increase in GPC-2 monomer fraction after sodium borohydride reduction. Values in parentheses are expressed as percent of GPC-1 dimer fraction.

[c]Peroxide values of 1062 for linolenate and 2000 for linoleate.

[d]Ferric chloride-ascorbic acid, room temperature (27).

lenate by HPLC, but considerable decomposition and depolymerization were experienced with microporous silica columns. These oxidative dimers of methyl linolenate apparently are much more labile than those previously separated by HPLC from autoxidized methyl linoleate (19). Better recoveries were obtained with reverse phase C_{18} columns, but the separations were not useful. The dimeric components in GPC-1 fractions were, therefore, characterized spectrally without further separation. UV analyses (methanol) showed major conjugated diene absorption at 234 nm and minor conjugated carbonyl absorption at 274 nm. IR analysis (CS_2) confirmed the UV analyses in showing bands due to *cis,trans* and *trans,trans* conjugated dienes (980, 950 cm^{-1}), conjugated carbonyl (1,725, 1,710 cm^{-1}), isolated *trans* (960 cm^{-1}), as well as peaks due to hydroxyl (3,600–3,150 cm^{-1}), olefinic (3,010 cm^{-1}), and ester carbonyl bonds (1,745 cm^{-1}).

The ^1H NMR spectrum of the GPC-1 fraction from methyl linolenate autoxidized to peroxide values of 119 and 1,060 showed resonance signals similar to those previously reported for a peroxide-linked linoleate dimer with conjugated diene components (3), hydroperoxy epidioxides and other secondary oxidation products of methyl linolenate (6). Shifts for protons of the peroxy methine group were found at 4.32 and 4.14 ppm, attributed to intermolecular peroxide cross-linkage between the monomeric units (3), at 4.48 ppm, due to the intramolecular peroxide of hydroperoxy epidioxide units (6), and at 3.61 and 3.71 ppm, due to ether linkage (Table 3). Other resonance peaks were observed for conjugated olefinic protons (5.42–5.72 ppm), isolated unsaturation (5.23–5.48 ppm), and methylene protons from epidioxide rings (2.80, 2.48 ppm) (6).

Conditions used for FABMS resulted in depolymerization of the dimer because the spectra obtained corresponded to the monomeric units. Molecular peaks for the GPC-1 fraction of autoxidized methyl linolenate (peroxide value 1,060) corresponded to the following monomeric units: molecular species, mass (relative abundance): monohydroperoxides, M, 324 (44); M-OH, 307 (44); M-OOH, 291 (91); dihydroperoxides, M-1, 355 (86); and hydroperoxy epidioxides, M-1, 355 (2.3), M-17, 339 (22%).

Further structural information was obtained on the monomeric units of the dimer fraction of autoxidized methyl linolenate (peroxide value 1,060) by capillary GC and GC-MS analyses after catalytic hydrogenation to the hydroxyesters followed by silylation to the TMS derivatives. Capillary GC analyses revealed the presence of 35% monohydroxy stearates (9- + 12- + 13-+ 16-isomers), 25% dihydroxy stearates (9,10-, 12,13-, 15,16-, 9,16-isomers), and 40% trihydroxy stearates (9,10,12- and 13,15,16-isomers). The isomeric distribution of the mono-hydroxystearates was similar to that of methyl linolenate hydroperoxides, with larger amounts of 9- and 16-isomers than the 12- and 13-isomers (33,34).

On the basis of previous work on the monohydroperoxides (33), and monomeric secondary products of methyl linoleate and linolenate (2,6), reduction and hydrogenation would produce monohydroxyesters from dimers with monohydroperoxide units, dihydroxyesters from dimers with dihydroperoxide units, and trihydroxyesters from dimers with hydroperoxy epidioxide units. Therefore, our results support structures for the dimeric fractions from methyl linolenate autoxidized at 40 C that are composed of mono-, dihydroperoxides,

TABLE 3

^{1}H NMR of GPC-1 Dimer Fractions from Decomposed Linolenate Oxidation Products

Oxidation samples	Shifts, ppm (Multiplicity[a], assignments)
Autoxidized linolenate (PV 119) (40 C)	6.90 (br, OH/OOH), 5.42-6.72 (m, CH=CH-CH=CH), 5.23-5.48 (m, CH=CH), 4.48 (m, CH-OO-CH), 4.14, 4.32 (m, CH-OO-), 3.67 (s, OCH_3), 3.61, 3.71 (s, CH-O-CH), 2.94 (t, C=C-CH_2-C=C), 2.80, 2.48 (m, CH_2-CH-OO-CH-), 2.30 (t, CH_2-C=O, CH_2-OO-), 2.01 (m, CH_2-CH=CH-), 1.1-1.8 (m, -CH_2-), 0.9-1.1 (t, CH_3)
Monohydroperoxides (40 C) (Fig. 3)	5.5-6.65 (m, CH=CH-CH=CH), 5.3-5.52 (m, CH=CH), 4.34 (m, CH-OO-), 3.67 (s, OCH_3), 3.64, 3.72 (s, CH-O-CH), 2.94 (t, C=C-CH_2-C=C), 2.80, 2.40 (m, CH_2-CH-OO-CH-), 2.30 (t, CH_2-C=O, CH_2-OO-), 2.05 (m, CH_2-CH=CH-), 1.2-1.8 (m, -CH_2-), 0.9-1.1 (t, CH_3)
Monohydroperoxides (150 C)	6.3-5.2 (m, CH=CH), 3.67 (s, OCH_3), 2.9 (t, C=C-CH_2-C=C), 2.32 (t, CH_2-C=O), 2.05 (m, CH_2-CH=CH-), 1.2-1.8 (m, -CH_2-), 0.96 (t, CH_3)
Monohydroperoxides (Fe-ascorbic)	6.3-5.2 (m, CH=CH), 4.40 (m, CH-OO-), 4.05, 4.32 (m, CH-OO-), 3.67 (s, OCH_3), 3.42, 3.71 (s, CH-O-CH), 2.92 (t, C=C-CH_2-CH=CH-), 2.78, 2.55 (m, CH_2-CH-OO-CH-), 2.30 (t, CH_2-C=O, CH_2-OO-), 2.03 (m, CH_2-CH=CH-), 1.2-1.8 (m, -CH_2-), 0.90 (t, CH_3)
Hydroperoxy epidioxides (40 C) (Fig. 4)	6.9 (s, OH/OOH), 5.42-6.08 (m, CH=CH-CH=CH), 5.3-5.4 (m, CH=CH), 4.80, 4.49 (m, CH-OO-CH), 4.13, 4.32 (m, CH-OO-), 3.67 (s, OCH_3), 3.61, 3.71 (s, CH-O-CH), 2.82, 2.48 (m, CH_2-CH-OO-CH-), 2.31 (t, CH_2-C=O CH_2-OO) 2.05, 2.19 (m, CH_2-CH=CH-), 1.1-1.8 (m, -CH_2-), 1.0 (t, CH_3)
Dihydroperoxides	7.6 (br, OH/OOH), 5.2-6.8 (m, CH=CH), 4.35 (m, CH-OO-), 4.05 (m, CH-OH/OOH), 3.67 (s, OCH_3), 3.42, 3.71 (s, CH-O-CH), 2.80, 2.40 (m, CH_2-CH-OO-CH-), 2.32 (t, CH_2-C=O, CH_2-OO-), 2.03 (m, CH_2-CH=CH-), 1.2-1.8 (m, -CH_2-), 0.95 (t, CH_3)

[a]Multiplicity: br, broad; s, singlet; t, triplet; m, multiplet.

and hydroperoxy epidioxides as monomeric units, with diene and carbonyl conjugation, and joined mainly with peroxide linkages. The predominant dimeric structures from autoxidized methyl linolenate are postulated on this basis in Figure 2.

Dimer structure A corresponds to two linolenate 9- or 16-hydroperoxide units with conjugated diene-triene systems joined by a peroxide linkage. The 12- and 13-hydroperoxide isomers are found in small concentrations in autoxidized linolenate because of their great tendency to cyclize into hydroperoxy epidioxides (6). Our finding of the same isomeric distribution from linolenate dimers after $NaBH_4$ reduction indicates that these internal hydroperoxide isomers tend to cyclize intramolecularly rather than to dimerize into compounds of structure A. Dimer structure B corresponds to one linolenate 9-/16-dihydroperoxide joined with a monohydroperoxide unit. Dimers involving the corresponding 12-/13-dihydroperoxides are less important because they are also found in small concentrations in the monomeric dihydroperoxides of methyl linolenate (6). Dimer structure C corresponds to two linolenate hydroperoxy epidioxide units. Dimer structure D is a combination of one hydroperoxy epidioxide unit with a monohydroperoxide, and dimer structure E corresponds to two dihydroperoxide units. Other dimer structures can, of course, include all combinations between mono-, dihydroperoxides, and hydroperoxy epidioxides.

Dimers from methyl linolenate monohydroperoxides. Pure methyl linolenate hydroperoxides were oxidatively polymerized under the same conditions as methyl linolenate at 40 C. Reverse-phase TLC (methanol) of the dimeric GPC-1 fraction showed two major UV-positive spots of R_f 0.78 and 0.69. UV spectra showed evidence for conjugated diene, conjugated carbonyl, and conjugated triene unsaturation with maxima at 233 nm (E_m

= 10,600), and 268 nm (E_m = 7,365). IR (CS_2) showed hydroxyl bands (3,620–3,200 cm^{-1}), conjugated carbonyl (1,720 cm^{-1}), *cis,trans* and *trans,trans* conjugated dienes (985 and 940 cm^{-1}), and isolated *trans* unsaturation (965 cm^{-1}). Capillary GC and GC-MS (after hydrogenation and silylation indicated the presence of 52% monohydroxy stearate (33.5% 9-OH, 17.7% 12-OH, 15.3% 13-OH, 33.5% 16-OH), 32% dihydroxy stearate (6.2% 11,13-, 7.3% 9,10-, 15.3% 12,16-, 14.2% 9,13-, 9.2% 11,12- and 11,13-, 11.0% 15,16-, and 36.9% 9,16-diOH), and 17% trihydroxy stearate (Table 4). The isomeric distribution of the monohydroxy stearate components is similar to that of the starting linolenate monohydroperoxides (33,34). FABMS of the dimers showed features for monohydroperoxides with M-1, 323 (59%), and hydroxy hydroperoxide with M-1, 339 (32%). The ^1H NMR spectrum (Fig. 3, Table 3) showed some of the same features as autoxidized methyl linolenate with much weaker signals due to hydroperoxy epidioxide components, as follows: resonance for conjugated olefins (5.5–6.65 ppm), isolated unsaturation (5.3–5.52), methine protons for a peroxide bridge (4.34 ppm) (Fig. 3). Very small peaks at 3.64 and 3.72 ppm on the shoulders of the methoxy singlet (3.67 ppm) can be attributed to the ether bridge. The results indicate dimer structures involving condensation of mainly monohydroperoxide, dihydroperoxide, and small amounts of epidioxy hydroperoxide units with either peroxide linkages (Structures A–E, Fig. 2) or ether linkages, conjugated diene, isolated unsaturation and methylene between two double bonds.

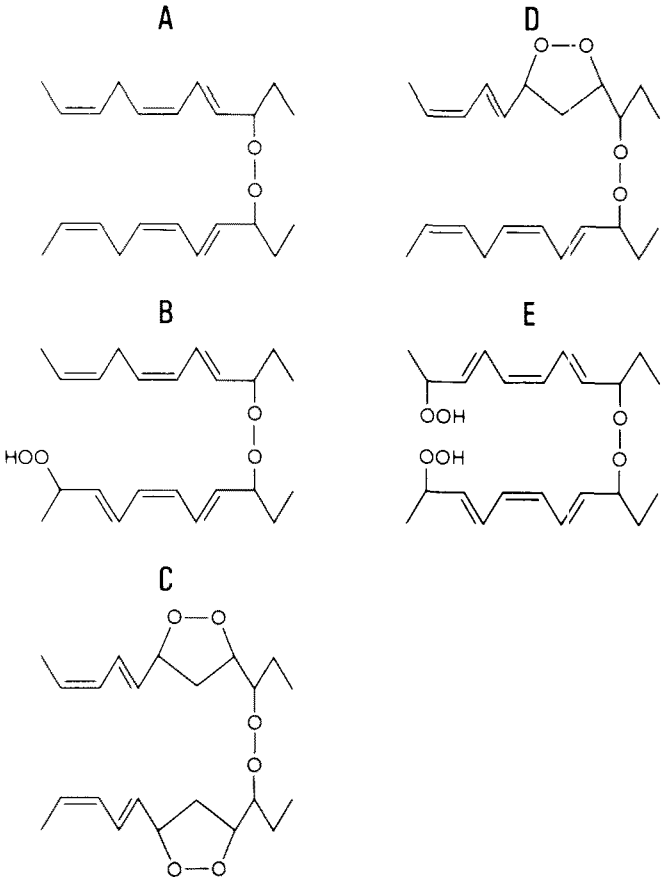

FIG. 2. Main structures of peroxide-linked dimers formed by autoxidation of methyl linolenate at 40 C.

TABLE 4

Capillary Gas Chromatographic Analysis of Hydrogenated Dimers (after silylation)

Oxidation samples	Decomposition conditions	Hydroxy stearates[a] Mono	Di	Tri
Autoxidized linolenate				
PV 119	40 C	67	16	17
PV 1062	40 C	35	25	40
Linolenate hydroperoxides	40 C	52	32	17
	Fe-ascorbic[b]	85	0	15
Hydroperoxy epidioxides	40 C	12	32	56
Dihydroperoxides	40 C	0	78	22

[a]As trimethylsilyl ethers; mono esters come from monohydroperoxides, di esters come from di-hydroperoxides and tri esters from hydroperoxy epidioxides (33).

[b]Ferric chloride-ascorbic acid, room temperature (27).

Oxidative polymerization of methyl linolenate monohydroperoxides at 150 C produced dimers that contained no peroxide linkage because the proportion of the GPC-1 fraction remained unchanged after $NaBH_4$ reduction (Table 2). The ^1H NMR spectrum supported the GPC analyses by showing the absence of resonance at 4.30–4.15 for peroxide linkage. There was no evidence for ether linkage on each side of the peak for the methoxy group at 3.67 ppm, in contrast to the oxidative dimers from methyl linolenate and the derived hydroperoxides prepared at 40 C (Table 3). The similarity of this NMR spectrum to that reported for thermal dimers of methyl linoleate (35) supports a carbon linkage in our linolenate dimers. The evidence for a carbon linkage was further supported by the resistance of the dimer to depolymerization by catalytic hydrogenation. The ^1H NMR, IR and UV spectra provided evidence for conjugated diene and isolated unsaturation, but no hydroxyl or hydroperoxyl absorption. Therefore, the dimeric structure involves condensation of two hydroperoxide units, such as A (Fig. 2), with carbon linkages instead of peroxide linkages.

The dimers produced by polymerization of methyl linolenate monohydroperoxides with ferric chloride and ascorbic acid at room temperature were very similar to those prepared at 40 C, except for a higher proportion of ether linkage (56.7 vs 28.3, Table 2). The IR, UV and ^1H NMR (Table 3) spectral data were very similar in showing the presence of conjugated diene, isolated olefin, hydroxyl or hydroperoxyl absorption, peroxyl and ether linkages. Capillary GC and GC-MS after hydrogenation and silylation produced mixtures of 85% monohydroxy stearate (32.6% 9-OH, 14.8% 12-OH, 14.8% 13-OH, 32.6% 16-OH), and 15% trihydroxy stearate (9,10,12- and 13,15,16-triOH in 1:1 ratio) (Table 4). The data are consistent with a mixture of dimeric structures containing monohydroperoxides

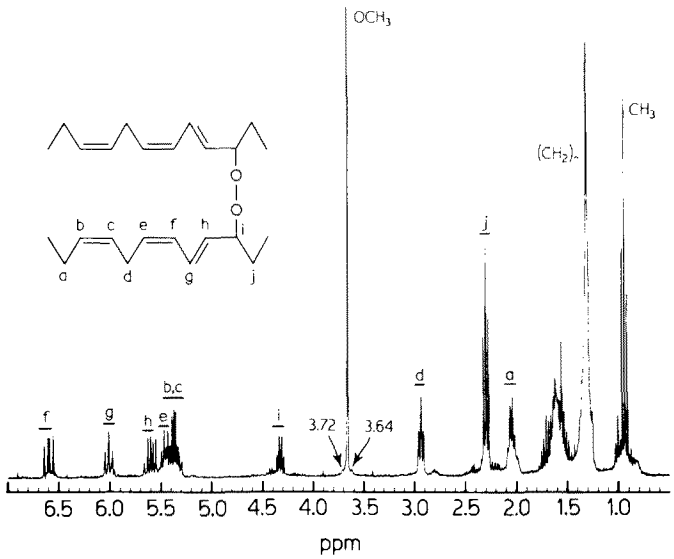

FIG. 3. ^{1}H NMR spectrum of dimer fraction from methyl linolenate mono-hydroperoxides polymerized in oxygen at 40 C.

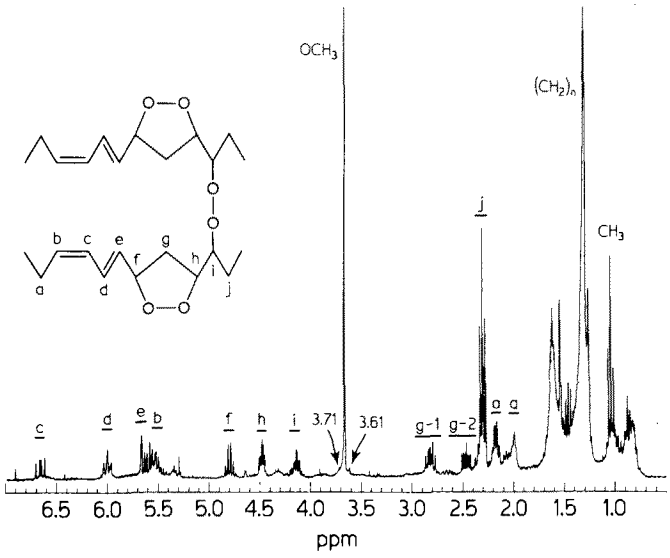

FIG. 4. ^{1}H NMR spectrum of dimer fraction from methyl linolenate hydroperoxy epidioxides polymerized in oxygen at 40 C.

and hydroperoxy epidioxides with either peroxide linkages, such as A, C and D (Fig. 2), or with ether linkages. A dimeric structure similar to A was also identified in methyl linoleate hydroperoxides decomposed in the presence of either a free radical initiator (3) or metal catalyst (22).

Dimers from methyl linolenate hydroperoxy epidioxides. The dimers produced by polymerization of methyl linolenate hydroperoxy epidioxides at 40 C were similar to those prepared from monohydroperoxides under the same conditions. The peroxide-linked dimers, contributing 71% of the total dimers (Table 2), consisted of 56% hydroperoxy epidioxides, 32% dihydroperoxides, and 12% monohydroperoxides (Table 4). Capillary GC analyses of the hydrogenated derivatives showed that dihydroxy stearates consisted of 10% 9,10-, 22% 9,16-isomers, and the trihydroxy stearates consisted of an equal mixture of 9,10,12- and 13,15,16-isomers. The IR, UV and ^{1}H NMR (Fig. 4, Table 3) spectral data were also similar in showing the presence of conjugated diene, isolated olefin, hydroxyl or hydroperoxyl absorption, peroxyl and ether linkages. Other NMR absorptions showed epidioxide methylene protons (2.82 and 2.48 ppm), cyclic peroxide methine protons (4.80 and 4.49 ppm), and methane proton of hydroxyl- or hydroperoxyl-bearing carbon (4.13 ppm) (Fig. 4). The data support a mixture of dimer structures B, C and D (Fig. 2).

Dimers from methyl linolenate dihydroperoxides. The dimers produced by polymerization of pure methyl linolenate dihydroperoxides at 40 C contained 78% dihydroperoxides and 22% hydroperoxy epidioxide components (Table 4). The proportion of peroxide linkage was 82% (Table 2). The IR, UV and ^{1}H NMR (Table 3) spectral data were similar to those of the hydroperoxy epidioxide dimers in showing the presence of conjugated diene, isolated olefin, hydroxyl or hydroperoxyl absorption, peroxyl and ether linkages. Capillary GC after hydrogenation showed the dihydroxy stearates to consist of 19.3% 12,13-, 33.2% 9,10-, 15.2% 15,16- and 32.3% 9,16-isomers, and the trihydroxy stearates of an equal mixture of 9,10,12-and 13,15,16-isomers. The data are consistent with a mixture of structures C and E (Fig. 2).

Hydroperoxy epidioxides and dihydroperoxides constitute the major secondary products of the autoxidation of methyl linolenate (6,8). This work showed that autoxidative dimeriztion under mild conditions (40 C) involves significant proportions of the 9- and 16-hydroperoxy epidioxides joined mainly by peroxide linkages (Structures A–E, Fig. 2). This dimerization can occur by termination reactions involving peroxyl (ROO•), alkyl (R•) or alkoxy (RO•) radicals (36). The preponderance of peroxy-linked dimers indicates that terminations involving peroxyl and alkyl radicals are the most important under mild conditions of autoxidation. At higher temperatures (150 C) more ether- and carbon-linked dimers were formed, and the termination reactions involving alkoxy and alkyl radicals become more important. The peroxide-linked dimers can be more readily cleaved thermally and may be important precursors of volatiles contributing to flavor deterioration of linolenate-containing fats (25).

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