Volatile Compounds from the Triacylglycerol of *cis,cis* 9,15-Linoleic Acid

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***cis,cis* 9,15-Linoleic acid, one of the isomers produced by partial hydrogenation of linolenic acid, may be a precursor of the hydrogenation odor observed during heating of partially hydrogenated soybean oil in air. Odors of hydrogenation, for example the melon odor, are associated with foods cooked in partially hydrogenated soybean oil that has undergone oxidative deterioration during heating. To better understand this oxidation mechanism, the volatiles from decomposition of the oxidation products of a synthesized model compound, tri *cis,cis* 9,15-linoleoylglycerol, heated in air at 192°C were collected, separated, quantitated by gas chromatography with flame ionization detection and identified by mass spectrometry. Major volatiles observed indicated that each of the isolated double bonds at carbons 9,10 and 15,16 isomerized to form an allylic radical. The allylic radical derived from the isolated double bond at carbons 9,10 reacted with oxygen to form the 8,9,10 and 11 monohydroperoxides. The allylic radical derived from the isolated double bond at carbons 15,16 produced the 14,15,16 and 17 monohydroperoxides. The 8,9,10 and 11 monohydroperoxides are the expected precursors of the following volatiles identified by mass spectrometry: 2,8-undecadienal (5.9%), 2,7-decadienal (7.8%), 6-nonenal (8.4%) and 5-octenal (1.5% of total volatiles), respectively. These four aldehydes are analogous to major volatiles obtained from thermal decomposition of autoxidized trioleoylglycerol. The 14,15,16 and 17 monohydroperoxides are the expected precursors of 2-pentenal (9.0%), 2-butenal (8.0%), propanal (12.1%) and ethanal (2.0% of total volatiles), respectively. Among the above volatiles, 6-nonenal (cucumber-melon) is reported to have the most intense odor associated with deteriorated partially hydrogenated soybean oil.**

KEY WORDS: 2,6-Nonadienal, 5-octenal, 6-nonenal, *cis,cis* 9,15-linoleic acid, hydrogenation odor, isolinoleic acid, partially hydrogenated soybean oil, tri *cis,cis* 9,15-linoleoylglycerol.

Linolenic acid (Ln) in soybean oil (SBO) has long been known to be readily oxidized during storage and heating of the oil and is an important precursor of off-flavors and odors (1–8). Lowering the Ln content of SBO by hydrogenation is the principal method used to stabilize SBO (4,5). However, there are isomeric dienes or isolinoleates, formed during hydrogenation of Ln (9–13) that are considered to be precursors of an undesirable odor (4,5,14–18) described as a "hydrogenation" or "hardened" odor. Keppler *et al.* (15,16) synthesized and oxidized three of the isomeric dienes with double bonds at carbons 7,15, 8,15 and 9,15 formed by catalytic hydrogenation of methyl linolenate. They concluded that 6-nonenal caused the hydrogenated odor. They also detected, among the volatiles of oxidized 8,15- and 9,15-isolinoleates, seven other aldehydes, which they did not identify but which may also contribute to the hydrogenation odor. Frankel (4,5) has reviewed research concerning monohydroperoxides and their volatile products found in abused, partially hydrogenated oils. He reported that nonenal, due to its low threshold value, may be the most important volatile (melon or cucumber odor) in the production of the hydrogenation odor (5). However, he mentioned many other volatiles that could be expected to be generated from the hydroperoxides of the 9,15- and 12,15-isolinoleic acids, including unsaturated aldehydes with low odor threshold values (5). A previous study of the autoxidation hydroperoxides of 9,15- and 12,15-isolinoleic acid methyl esters listed several hydroperoxides, including the 10-hydroperoxide, which is the probable precursor of the aldehyde *cis* 6-nonenal (melon odor) (17). The results of the study suggested that the autoxidation mechanism for the isolinoleic esters with isolated double bonds was analogous to the autoxidation mechanism for an isolated double bond as in methyl oleate. Moreover, the double bond closer to the terminal methyl group produced more total hydroperoxides than the double bond closer to the methyl ester group for the isolinoleates.

We investigated the oxidation of a model triacylglycerol of *cis,cis* 9,15-linoleic acid, which is more closely related than the previously studied methyl ester (17) to the triacylglycerols involved in the formation of hydrogenated odor in partially hydrogenated SBO. A microroom technique, previously reported for analysis of volatiles from heated triacylglycerols (19–22) was used to investigate the volatiles generated from tri *cis,cis* 9,15-linoleoylglycerol, a triglyceride of an isomeric fatty acid generated during catalytic partial hydrogenation of soybean oil.

EXPERIMENTAL PROCEDURES

Tri cis,cis *9,15-linoleoylglycerol.* Preparation and purification of *cis,cis* 9,15-linolenate has been described by Frankel (17). The triglyceride was prepared from the 9,15-acid chloride mixed with glycerol and pyridine. The crude product was isolated and purified by alumina followed by silica gel liquid-column chromatography. Thin-layer chromatography on silica of the chromatographed product developed with petroleum ether/diethyl ether/acetic acid (70:25:5, vol/vol/vol) indicated no mono- or diacylglycerol present. Fatty acid analysis by gas-liquid chromatography/mass spectrometry of the purified tri *cis,cis* 9,15-linoleoylglycerol, after transmethylation, showed 99.3% methyl 9,15-linoleate, 0.2% methyl linolenate and trace amounts of other C-18 methyl esters.

Volatiles collection and analysis. Specific details of the volatiles collection and analysis procedure have been reported (19–22). Briefly, 2 mL of the tri *cis,cis* 9,15-linoleoylglycerol was heated to 192°C in a stainless steel container (microroom). During a subsequent 10-min heating period, volatiles generated from the hot oil were continuously wept out of the microroom with air and collected directly on a gas chromatography column (glass 14 ft × 4 mm i.d. column, packed with 10% OV-17 on chromosorb

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G) (Supelco, Bellefonte, PA) cooled to −60°C. The volatiles were separated by temperature programming to column to 250°C at 2°C/min. The column effluent was split three ways for simultaneous monitoring: to a flame ionization detector for quantitation of volatile components; to a mass spectrometer (MS) (double-focussing; magnetic scanning, set to scan M/Z 10–450 every 9 s) for volatile identification by comparison to standard spectra; and to a sniffing port for odor identification. Output from the MS was stored in computer data files for later processing. When applicable, mass spectrometric identification of volatile compounds, matched by computer with those of library reference compounds, were also confirmed by gas chromatography (GC) retention data. Because several compounds lacked a library reference, tentative identifications were based on interpretation of their mass spectra and comparison with the spectra of known compounds. Reference compounds used for GC retention data were purchased from Bedoukian Research Inc. (Danbury, CT).

RESULTS

The gas chromatogram of the resolved volatile components generated from tri *cis,cis* 9,15-linoleoylglycerol heated in air at 192°C in a microroom is presented in Figure 1. Twenty volatiles, representing 80% of the total gas chromatogram peak area, were identified by MS through library spectra and reference compounds, and by GC retention times or elution temperatures of reference compounds. Relative percent composition (peak areas) for the volatiles identified in Figure 1 are presented in Table 1. In addition to 6-nonenal (8.4%), the volatiles 5-octenal (1.5%) and 2,6-nonadienal (0.3%) also had melon or cucumber odors, characteristic of volatiles contributing to the hydrogenation odor (4,5).

DISCUSSION

The isolated double bonds in the tri *cis,cis* 9,15-linoleoylglycerol should oxidize to produce hydroperoxides according to a scheme similar to a monoenoic acid like oleic (4–6, 17). The monoenoic acid autoxidation mechanism involves hydrogen abstraction from the methylene carbons adjacent to each double bond to form two resonance propane radicals per double bond (4–6), as depicted in Figure 2. Oxygen addition to the ends of the resonance system plus hydrogen produces four hydroperoxides. Thus, the 9,15-linoleic acids attached to the glycerol moiety would be expected to produce 8 allylic monohydroperoxides (Fig. 2) (17). These hydroperoxides can decompose thermally (5–7) to generate volatile compounds according to the schemes presented for 8,9,10 and 11 monohydroperoxides (Fig. 3) and 14,15,16 and 17 monohydroperoxides (Fig. 4).

The monohydroperoxide decomposition schemes presented in Figures 3 and 4 are based on the general assumption (5) that, upon initial cleavage, hydroxyl and alkoxy radicals are produced. The alkoxy radical goes through a carbon-carbon cleavage on each side of the carbon bearing the oxygen atom. With the triacylglycerol, thermal decomposition produced one nonvolatile glyceride-bound fragment and volatile fragments. For example, in Figure 3 for the 10-monohydroperoxide, cleavage A generates a C-9 free fragment containing oxygen, which upon rearrangement produced the 6-nonenal. Cleavage B, between the 10 and 11 carbons, would produce a glyceride-bound fragment and a 8-carbon hydrocarbon radical. This radical

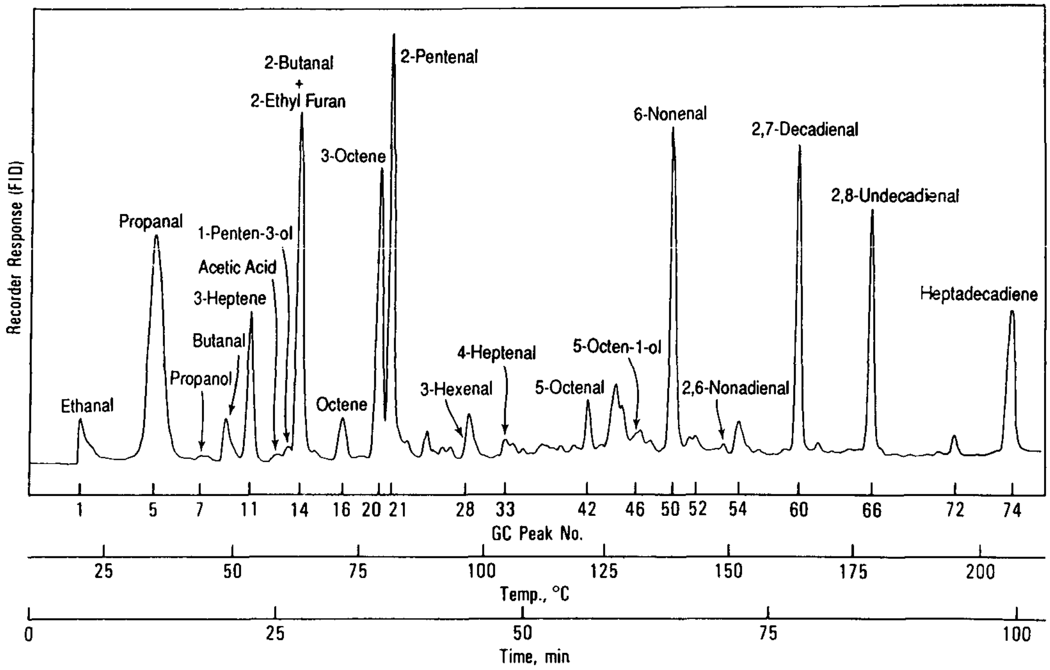


FIG. 1. Gas chromatogram of volatiles generated from heated (192°C) tri *cis,cis* 9,15-linoleoylglycerol. Volatiles collected at −60°C on 10% OV-17 on Chromosorb G column (glass, 6.8 m × 4 mm i.d.) were eluted by temperature programming to 250°C at 2°C/min, and subsequently noted by a flame-ionization detector (FID). GC, gas chromatography.

TABLE 1

Volatiles from Tri *cis,cis* 9,15-Linoleoylglycerol[a,b] Heated in Air at 192°C (Fig. 1)[c]

Volatile	Possible origin (Figs. 3,4) -OOH	GC FID relative percent	Hydrogenation odors
Ethanal	17	2.0	
Propanal	16	12.1	
Propanol		0.2	
Butanal	14	1.2	
3-Heptene	11	4.8	
Acetic acid		0.3	
1-Penten-3-01		0.4	
2-Butenal + 2-ethylfuran	14	10.2	
Octene (isomers)		1.5	
3-Octene	10	7.0	
2-Pentenal	15	9.0	
3-Hexenal		0.7	
4-Heptenal		0.6	
5-Octenal	11	1.5	Cantaloupe, watermelon rind
5-Octen-1-01		0.4	
6-Nonenal	10	8.4	Cucumber, watermelon rind
2,6-Nonadienal		0.3	Cucumber
2,7-Decadienal	9	7.8	
2,8-Undecadienal	8	5.9	
Heptadecadiene	Parent fatty acid	4.8	

[a] Preparation of the triacylglycerol and quantitative identification and odor analysis of the volatiles as presented in the Experimental Procedures section.

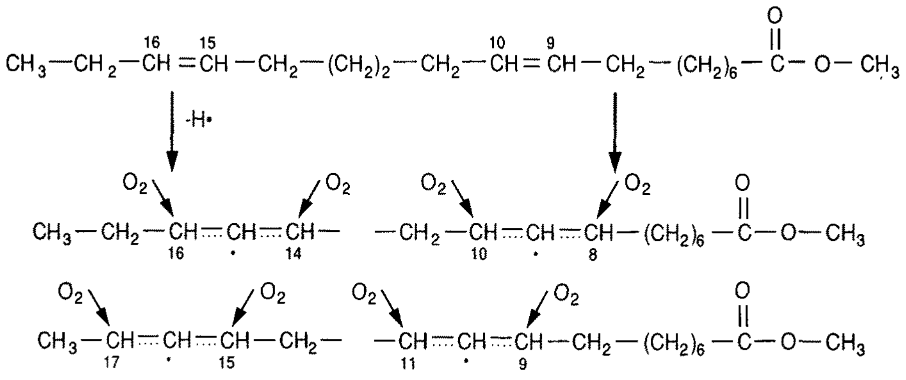
[b] Five *cis,cis* 9,15-linoleoylglycerol samples were oxidized and volatiles were analyzed.

[c] Abbreviations: GC, gas chromatography; FID, flame-ionization detector.

could react with a hydrogen radical to form the 3-octene or with a hydroxy radical to form 5-octen-1-ol, both identified in Figure 1 and listed in Table 1. In addition, the C-8 radical could further react with oxygen to form other compounds. Similar processes shown in schemes in Figures 3 and 4 can account for many of the volatiles listed in Table 1.

It may appear contradictory that, of the eight monohydroperoxides of 9,15-linoleic acid, the 17-monohydroperoxide was most abundant (22%, Fig. 2) (17), while its predicted decomposition product (ethanal, Fig. 4) was observed only at the 2% level (Table 1). This may indicate that the 17-monohydroperoxide was more stable or that other reactions occurred to prevent ethanal accumulation. On the other hand, propanal (Fig. 4) from the 16-monohydroperoxide and 6-nonenal from the 10-monohydroperoxides (Fig. 3) were observed in satisfactory amounts. Apparently, these volatiles are stable enough to accumulate.

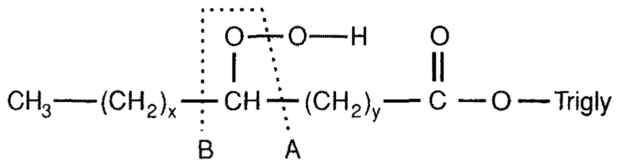
The major GC peaks shown in Figure 1 are associated with the predicted volatiles (Figs. 3 and 4) from the eight monohydroperoxides previously identified for oxidation of methyl *cis,cis* 9,15-linoleic acid (Fig. 2) (17). In general, cleavage A of a monohydroperoxide (Figs. 3 and 4) forms an oxygen-containing free fragment, which rearranges to an aldehyde to account for the volatiles in Table 1. Cleavage B forms the hydrocarbon radical that can react with a hydrogen or hydroxyl radical to form the hydrocarbon and alcohol volatiles respectively listed in Table 1. Cleavage A products, which are simple cleavage compounds, are likely to be more abundant than B cleavage compounds because these latter compounds also require a second reaction (•H or •OH) for completion. Heptadecadiene was probably not derived from a monohydroperoxide, but was probably a thermal decomposition product at the fatty acid carbon chain (19,20). About 13% of the identified volatiles were most likely cleavage B products.



Composition of Monohydroperoxides
Double Bond

15-16		9-10	
HP	%	HP	%
14	11	8	11
15	8	9	12
16	15	10	11
17	22	11	10

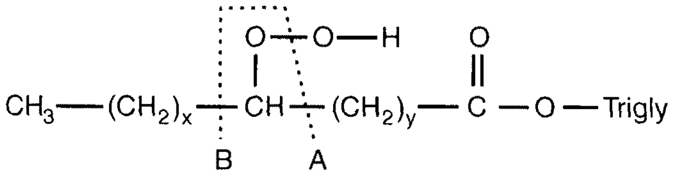
FIG. 2. Mechanistic scheme for the formation of monohydroperoxides from methyl *cis,cis* 9,15-linoleate (16). Percentage data obtained by gas chromatography-mass spectrometry of the silylated hydrogenated methyl esters of the triacyl glycerols. HP, hydroperoxides.



$CH_3-(CH_2)_x-CH(O-O-H)-(CH_2)_y-C(=O)-O-Trigly$

Hydroperoxide Position	A-Cleavage	B-Cleavage	
		•H	•OH
8-00H	2,8-Undecadienal (5.9%)	1,7-Decadiene (ND)	7-Decenal (ND)
9-00H	2,7-Decadienal (7.8%)	1,6-Nonadiene (ND)	6-Nonenal (?)
10-00H	6-Nonenal (8.4%)	3-Octene (7.0%)	5-Octen-1-ol (0.4%)
11-00H	5-Octenal (1.5%)	3-Heptene (4.8%)	4-Hepten-1-ol (ND)

FIG. 3. Mechanistic scheme for hydroperoxide thermal decomposition and composition of volatiles generated from 8,9,10,11 monohydroperoxides of tri *cis,cis* 9,15-linoleoylglycerol. Percentage data of total volatiles is from Table 1. ND, none detected.



$CH_3-(CH_2)_x-CH(O-O-H)-(CH_2)_y-C(=O)-O-Trigly$

Hydroperoxide Position	A-Cleavage	B-Cleavage	
		•H	•OH
14-OOH	2-Pentenal (9.0%)	1-Butene (ND)	Butanal (1.2%)
15-OOH	2-Butenal (8.2%)	1-Propene (ND)	Propanal (?)
16-OOH	Propanal (12.1%)	Ethane (ND)	Ethanol (ND)
17-OOH	Ethanal (2.0%)	Methane (ND)	Methanol (ND)

FIG. 4. Mechanistic scheme for hydroperoxide thermal decomposition and composition of volatiles generated from 14,15,16,17 monohydroperoxides of tri *cis,cis* 9,15-linoleoyglycerol. Percentage data of total volatiles is from Table 1. ND, none detected.

In this study, the identifications of 2,7-decadienal and the 2,8-undecadienals were only tentative because no authentic compounds were available. The origin of 2-ethylfuran, which co-eluted with 2-butenal, was unknown. However, mass chromatography (19) showed that 2-ethylfuran represented 18.0%, and 2-butenal, 82.0% of the peak area.

The odor descriptions were obtained by two people sniffing at the GC exit port. As indicated in Table 1, compounds that have a double bond at carbons 2,5 and 6 produced a cucumber or melon response, which may contribute to the hydrogenation odor of partially hydrogenated SBO. However, *trans* 6-nonenal has one of the lowest known odor thresholds, and its odor may mask that of other volatiles (5). The 6-nonenal, along with 5-octenal and 2,6-nonadienal, may account for the hydrogenation odor observed in deteriorated partially hydrogenated soybean oil.

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