Characterization of Nonvolatile Compounds Formed during Thermal Oxidation of 1-Linoleyl-2,3 Distearin

III. Evidence for Presence of Dimeric Fatty Acids

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ABSTRACT

Pure 1-linoleyl-2,3-distearin, which had been subjected to thermal oxidation at 200 C, was separated into its polar fatty acid fraction by solvent partitioning. Further fractionation was achieved via thin layer chromatography and partition column chromatography. These data, as well as data obtained from combined gas chromatography-mass spectrometry, indicated the presence of dimeric compounds.

INTRODUCTION

In contrast to the free radical mechanisms operative during autoxidation, the formation of high mol wt products during thermal polymerization proceeds primarily through Diels-Alder addition (1-3).

The presence of high mol wt polymeric products in thermally oxidized oils is well documented. For example, polymeric fractions isolated from corn oil oxidized at 200 C ranged in mol wt from 692-1600 (4). More recently, Barrett and Henry (5) have presented chromatographic and spectral evidence for the presence of dimers in cottonseed oil, which had been used for frying. In addition, Firestone also has presented chromatographic evidence for the presence of dimeric materials in cottonseed oil which had been heated at 225 C for 195 hr (6). It would be of interest to ascertain the formation of such dimeric products under less strenuous conditions and using a model triglyceride similar to those found in natural oils. In the present study both chromatographic and mass spectral evidence is presented for the formation of dimers from the model compound, 1-linoleyl-2,3-distearin, under such conditions.

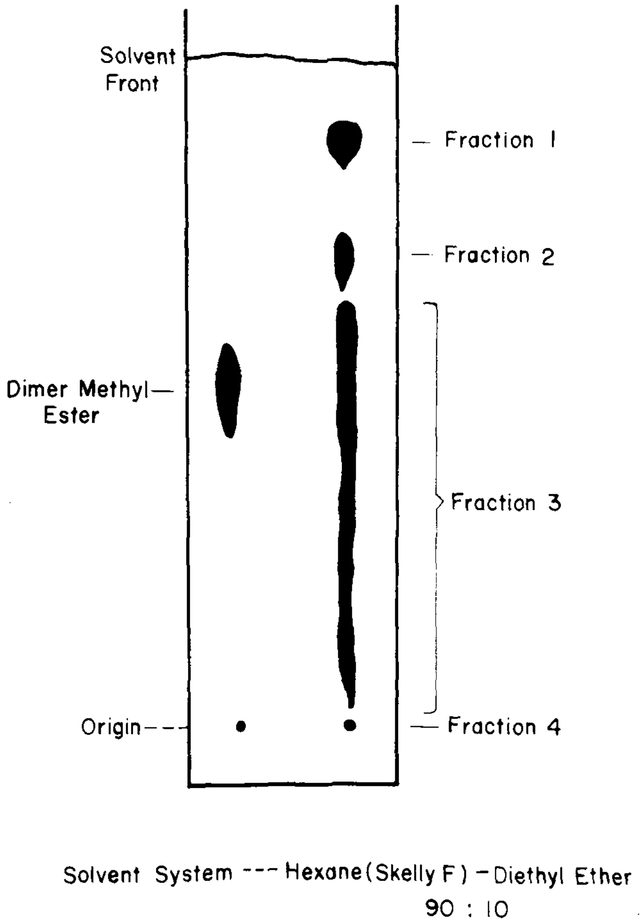

FIG. 1. Thin layer chromatogram of the polar fraction and dimer methyl ester. Solvent system: hexane (Skelly F)-diethyl ether 90:10.

EXPERIMENTAL PROCEDURES

Synthetic 1-linoleyl-2,3-distearin prepared, as previously described (7), was thermally oxidized at 200 C for 24 hr in the presence of air (0.10 ml/min/g). The resulting material was saponified, the nonacidic components removed, and the oxidized fatty acids partitioned between equilibrated 80% ethanol and redistilled Skelly Solve F to yield a polar and nonpolar fatty acid fraction (8).

The oxidized fatty acid fraction was converted to its corresponding methyl esters (8) and subjected to further separation by thin layer chromatography (TLC) using a solvent system of Skelly Solve F (hexane)-diethyl ether (90/10) (9).

Gas chromatography (GLC) was accomplished using a glass or stainless steel column 1/8 in. x 2.5 ft packed with 3% OV-1 coated on 80-100 mesh Chromasorb W (aw-dcms) (Supelco, Inc.). Carrier gas flow rates were 30 ml/min. The instruments used were a Barber Colman Model 10 with a β-ionization (^{3}H-Diode) detector and Aerograph Model 1200 equipped with a flame detector. Gas chromatography-mass spectrometry was carried out, as previously described (10).

RESULTS

TLC of the methyl esters from the polar fraction of fatty acids yielded four major fractions (Fig. 1). Fractions 1, 2, and a portion of fraction 3 have been characterized

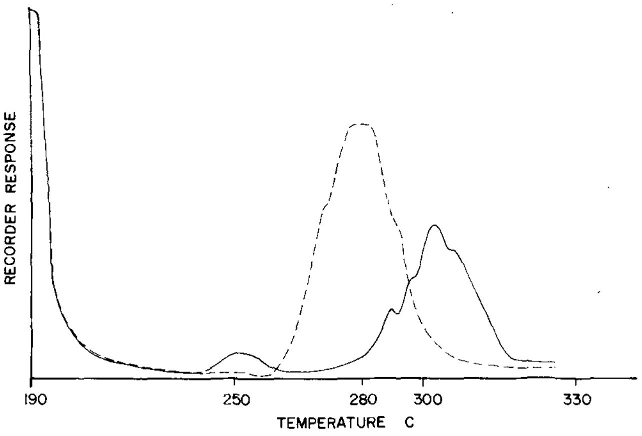

FIG. 2. Gas chromatogram of dimer fraction (———) and dimer standard (- - - -).

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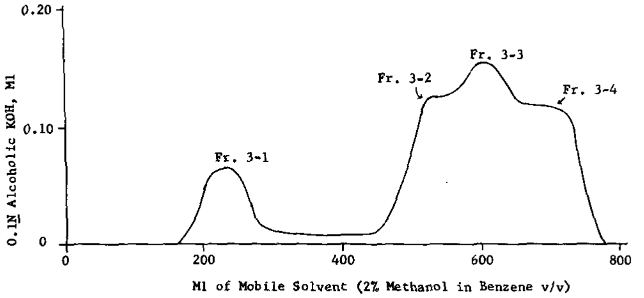


FIG. 3. Elution pattern from liquid partition chromatogram of polar fatty acids.

previously (7,8). These fractions were composed of unoxidized fatty acid esters, isomeric methyl keto-octadecanoates, methyl undecane-1, 11-dioate, and a mixture of isomeric C_{18} aromatic methyl esters. In addition to these materials, the long streak found as fraction 3 (Fig. 1) seemed to contain dimeric type methyl esters based upon the simultaneous behavior of a standard dimer ester on TLC. Furthermore, when this fraction (fraction 3, Fig. 1) was subjected to temperature programed gas chromatography from 150-325 C using an 18 in. x 1/8 in. SE-30 column, a series of poorly resolved peaks were eluted from 280-310 C. When a C_{36} dimer methyl ester was injected onto the same column under identical conditions, a series of ill-defined peaks eluting between 260-300 C was observed (Fig. 2).

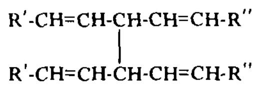
Further purification of the dimer fraction (designated Compound VI) was attempted using the liquid partition chromatographic method developed by Frankel (11) for the separation of dimeric and polymeric fatty acids. The free fatty acids corresponding to the crude dimer fraction (fraction 3, Fig. 1) were applied to the column and individual fractions titrated to a thymol blue end point with 0.01 n KOH to yield the dimeric fractions (Fig. 3). The fractions designated 3-2 to 3-4 were pooled and subjected to high temperature gas chromatography on an 18 in. x 1/8 in. SE-30 column, programed between 150-325 C at 6 C/min. The chromatogram obtained is shown in Figure 2 and is quite similar to that obtained from a known dimer standard.

Since the dimeric material appeared in the crude polar fatty acid mixture at ca. the 2% level, further attempts were made to concentrate the elutable material using preparative TLC. It was found that the dimeric fraction could not be separated conveniently from other polar material by this method. Therefore, direct introduction into the mass spectrometer of the effluent from the gas chromatograph was employed to characterize partially the dimeric fraction. When the crude mixture containing the dimer fraction was treated in this way under the GLC conditions described earlier and programed from 190-310 C, a series of four partially resolved peaks, which eluted between 285-310 C were observed (Fig. 2).

The mass spectrum (20 eV) (Fig. 4), which was recorded at the apex of the largest (third) component, exhibited a distribution of high mass ions ranging in mass from 581-591. These were interpreted as molecular ions, or ions formed by the abstraction of hydrogen atoms from molecular ions. Peaks, representative of the loss of a CH_3O fragment from molecular ions, were observed at masses 557-559. The base peak, which occurred at m/e 433, may be attributed to a loss of a $(CH_2)_7CO_2CH_3$ fragment from the m/e 590 ion. Peaks adjacent to the base peak were assigned to the loss of the same fragment from other molecular ions. Additional peaks attributed to the loss of ester containing fragments from molecular ions, appeared as distributions around peaks at m/e 447, M - $(CH_2)_6CO_2CH_3$; 419, M - $(CH_2)_8CO_2CH_3$; 407, M - $(CH_2)_9CO_2CH_3$-2H; and 393, M - $(CH_2)_{10}CO_2CH_3$-2H. Peaks tentatively assigned to the loss of hydrocarbon fragments from molecular ions, appeared as distributions around peaks at m/e 533, M - $(CH_2)_3CH_3$; 519, M - $(CH_2)_4CH_3$; 505, M - $(CH_2)_5CH_3$; 491, M - $(CH_2)_6CH_3$; and 477, M - $(CH_2)_7CH_3$. Finally, the peaks distributed around m/e 295 were assigned to M/2 ions.

The above data indicated that "Compound VI" was similar in structure to the dimeric C_{36} methyl esters previously reported by Wheeler and White as occurring in thermally polymerized linoleate (12). Three observations supported this view. First, the unknown eluted from a GLC column in the same temperature range as a known dimer mixture. Second, a mol wt of 586-590 is indicative of a dimer methyl ester. Lastly, the presence of an intense M/2 peak suggests a dimer structure (12).

The structures of two types of dimers originating from methyl linoleate have been shown previously in the literature. The first is a noncyclic dehydro-dimer, which is formed through free radical reactions (13). Abstraction of a proton from carbon number 11 of methyl linoleate, results in the formation of a resonance hybrid of three radical species (13). There could be six possible isomeric dimers formed through the coupling of the appropriate radical species. The structure of one noncyclic dehydro-dimer is represented below:



$$\begin{array}{l}\text{R}'\text{-CH=CH-CH-CH=CH-R}''\\ \phantom{\text{R}'\text{-CH=CH-}}|\\ \text{R}'\text{-CH=CH-CH-CH=CH-R}''\end{array}$$

Preferential loss of $CH_3(CH_2)_4$ and $(CH_2)_7CO_2CH_3$ fragments would be expected from the mass spectral fragmentation of these linoleate dehydro-dimers.

The second type of established dimer is the Diels-Alder

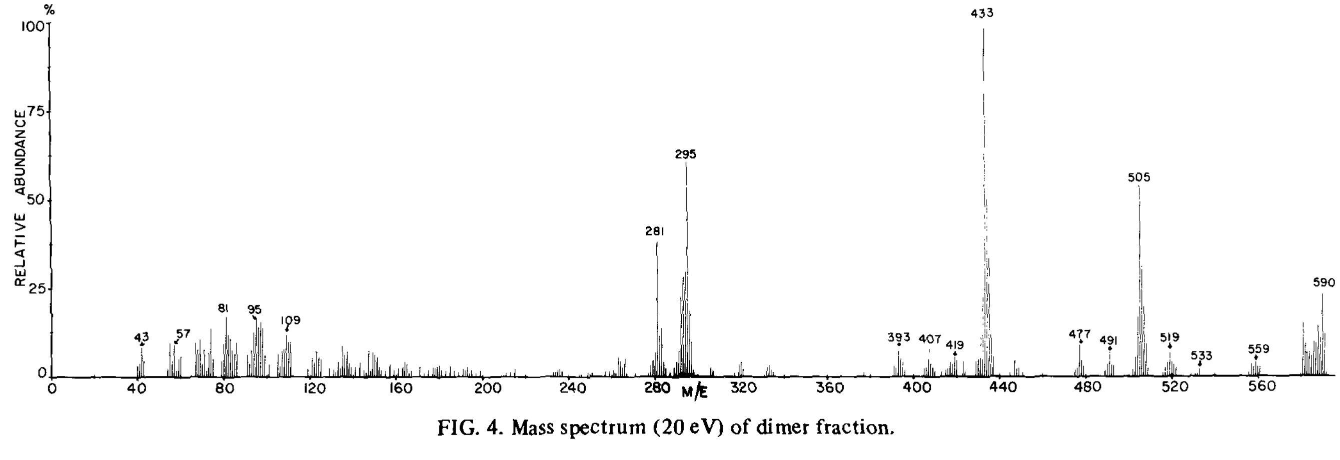
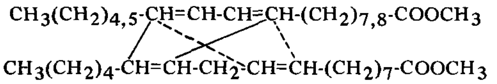


FIG. 4. Mass spectrum (20 eV) of dimer fraction.

structure which results from the 1,4-addition of one molecule of normal linoleate to a molecule of 9,11 or 10,12 conjugated linoleate (12):



Preferential loss of $CH_3(CH_2)_{4,5}$ and $(CH_2)_{7,8}COOCH_3$ fragments would be expected from the mass spectral fragmentation of these cyclic dimers (12).

The intense peaks in the mass spectrum of "Compound VI," which were observed for the losses of $CH_3(CH_2)_5$ and $(CH_2)_8CO_2CH_3$ fragments from molecular ions, suggest the presence of cyclic dimers. Furthermore, the loss of the hydrocarbon and ester containing fragments from molecular ions, which were observed in the mass spectrum of "Compound VI," have been observed in the spectrum of a Diels-Alder dimer from linoleate (12). An M/2 peak also is found in the spectra of both dehydro- and cyclic dimers (9,12,14,15). Therefore, no structural information was obtained by considering the intensities of the M/2 peaks. The molecular ion at mass 590 must contain either one ring and one double bond or two double bonds. Moreover, the spectra of cyclic linoleate dimers have characteristic masses at 586 and 588 (12). Thus, the presence of dehydro-dimers in the mixture, designated as "Compound VI," also was suggested. A linoleate dimer of mass 590 was proposed by Rushman (16), but no structure for this dimer was suggested.

The minor components of "Compound VI" (Fig. 2) also were examined by mass spectrometry. The only observable differences between the spectra of these components and that of the most abundant component were in the intensities and the distribution of the molecular ions. The two components which eluted prior to the largest component, exhibited distributions of peaks in the mass spectrum from m/e 582-586. The component, which eluted after the largest component, contained a distribution of peaks from 586-590 and is similar to the ion distribution reported by others (17).

The lack of further chemical and spectral evidence and the presence of many isomeric structures make it impossible to draw definitive structures for all the components of "Compound VI." However, it appears that this fraction is composed of both cyclic and noncyclic dimer species which differ in mol wt but which have similar structures.

ACKNOWLEDGMENTS

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