

The Autoxidation of Highly Unsaturated Fatty Acids: Methyl 4,7,10,13,16,19-Docosahexaenoate

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ABSTRACT

Methyl docosahexaenoate was selected as a model system for studying the autoxidation of highly unsaturated fatty acids. The ester was oxidized at 50 C for 28 hr and the volatiles collected by high vacuum distillation and fractionated by gas chromatography. A number of aldehydes, aldehyde esters, hydrocarbons and methyl esters were identified. Mechanisms are proposed for the formation of these compounds.

INTRODUCTION

The oxidation of highly unsaturated fatty acids, such as those abundant in marine oils, is more rapid and more complicated than that of the mono- or diunsaturated acids. However detailed information regarding oxidative mechanisms of nonconjugated fatty acids containing more than three double bonds is scarce. Various workers have oxidized fish oil methyl esters under a variety of conditions and determined the increase in peroxide value and conjugation (1-3). The formation of both mono- and dihydroperoxides was reported with the functional groups presumably located on the α -methylene carbons (4,5). Aldehydes appeared to be the major class of compounds produced from the oxidation of fish oil or fish oil methyl esters (7,8).

In this study the autoxidation of highly unsaturated fatty acids is investigated by examining the volatile compounds formed upon oxidation of methyl docosahexaenoate. Only aliphatic aldehydes, aldehyde esters, methyl esters and hydrocarbons are reported here. Identification of several compounds belonging to other classes will be presented in a later publication.

EXPERIMENTAL PROCEDURES

Materials

Methyl 4,7,10,13,16,19-docosahexaenoate (99% purity) was obtained from Hormel Institute, Austin, Minnesota. The methyl ester was further purified by stripping under vacuum (10^{-3} torr) at 75 C until the distillate collected on a cold finger showed no impurities when analyzed by gas chromatography.

Chemicals for reference standards were obtained commercially. Certain aldehydes and aldehyde esters not available commercially were synthesized in the laboratory. Details of synthesis and mass spectral fragmentation patterns of semialdehyde esters are published elsewhere (9). Authentic samples of 2,4-heptadienal, 2,4-nonadienal and 2,4-decadienal were obtained through the courtesy of C.B. Whitlock, University of Massachusetts, Amherst.

Oxidation

Two gram aliquots of the ester were placed in a 100 ml round bottom flask and maintained at 50 C for a period of 28 hr. During this period the sample was exposed to the atmosphere and continuously stirred with the aid of a magnetic stirrer. Precaution was taken to minimize direct lighting.

Analysis of Oxidation Products

The volatile compounds were collected in a cold finger-high vacuum system as described by Nawar et al. (10). The condensate was rinsed from the cold finger with 15 ml of pentane onto 5 g of silica gel. The mixture was stirred for 10 min and filtered, the residue washed with an additional 5 ml of pentane and the filtrates combined to constitute the "nonoxygenated" fraction. After washing with small quantities of pentane to remove traces of nonoxygenated components, the silica gel was extracted twice with 10 ml diethyl ether and the extracts combined to give the "oxygenated" fraction. Both fractions were concentrated under a stream of nitrogen to approximately 0.2 ml. It should be pointed out that some loss of the lower boiling compounds may occur during the concentration step. The pentane extract was analyzed on a 6 ft x 1/8 in. OD 10% silicone rubber SE-30 gas chromatographic column connected to a Hitachi Perkin-Elmer RMU-6A mass spectrometer (GC-MS). The ether extract was separated on a 12 ft x 1/8 in. OD Carbowax 20 M column equipped with a splitter. Twenty-five fractions were collected in capillary tubes which were inserted into the exit port and cooled with dry ice. Each fraction was then rechromatographed on the SE-30 column and analyzed in the coupled GC-MS system. Whenever feasible individual peaks were also trapped separately for IR analysis.

RESULTS

Eighty-seven components were detected and analyzed in the oxygenated fraction and 16 in the nonoxygenated fraction of oxidized methyl docosahexaenoate. In this paper only certain major components are reported. The remaining compounds will be the subject of another presentation.

Aldehyde Esters

Seven aldehyde esters were identified. They are: 3-formyl methyl propanoate (C4:0); 4-formyl methyl butanoate (C5:0); 6-formyl methyl 4-hexenoate (C7:1); 5-formyl methyl 4-pentenoate (C6:1); 7-formyl methyl 4,6-heptadienoate (C8:2); 9-formyl methyl nonadienoate (C10:2); 12-formyl methyl dodecatrienoate (C13:3). The retention time data on the Carbowax and SE-30 columns for the C4:0, C7:1, C10:2 and C13:3 compounds as well as infrared and mass spectra were identical to those of the authentic compounds synthesized in our laboratory. The infrared and mass spectra of the C6:1 and C8:2 were similar to those of the C7:1 and C10:2, respectively. The C5:0 was only tentatively identified on the basis of its mass spectral pattern.

Aldehydes

Ten aliphatic aldehydes were identified in the oxidized ester. They are: propanal, hexanal, 3-hexenal; 2,4-heptadienal; 3,6-nonadienal; 2,4-decadienal; decatrienal; 3,6,9-dodecatrienal, tridecatetraenal; 3,6,9,12-pentadecatetraenal. The retention time and mass spectral characteristics of propanal, hexanal, 3-hexenal, 2,4-heptadienal, 2,4-decadienal, 3,6,9-dodecatrienal and 3,6,9,12-pentadecatetraenal were identical to those of the standard reference compounds.

The component identified as 2,6-nonadienal gave the

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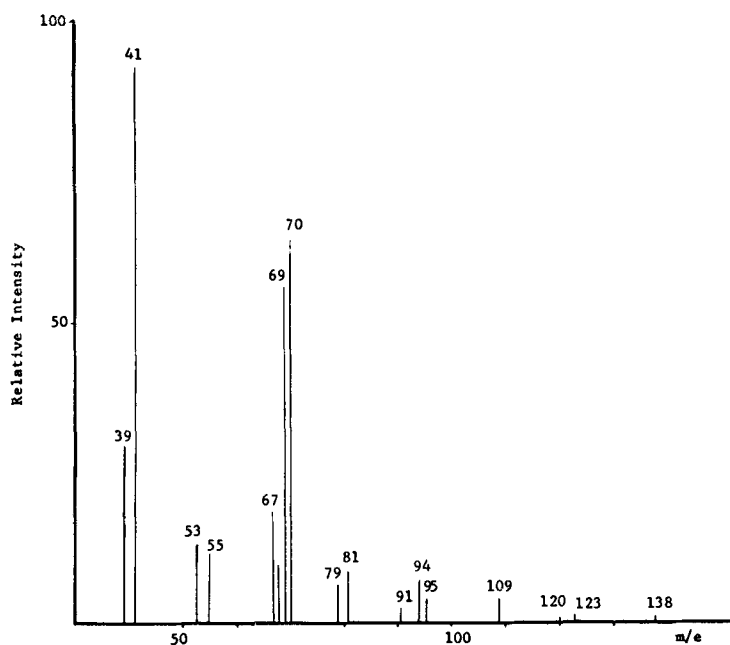


FIG. 1. Mass spectrum of compound identified as 2,6-nonadienal.

same gas chromatographic (GC) retention times as those obtained from authentic samples of 2,4-nonadienal and 3,6-nonadienal but the mass spectral patterns of the three isomers were different. The much greater intensity of the ions at m/e 70 and m/e 69 in case of the former compound (Fig. 1) would be explained by a 4-5 bond cleavage of a 2,6-dienal. Similar behavior was reported by Creveling and Jennings (11) for the fragmentation of methyl and ethyl 2,6-dodecadienoates. In the spectra of both of these compounds the base peaks correspond to the ions formed by 4-5 cleavage and hydrogen rearrangement with the charge retained on the oxygenated functional group. Further supporting evidence for this conclusion is the data on the 2,6-nonadienal published by Seifert and Buttery (12), in which the base peak was at m/e 41 and the ions listed in order of intensity were m/e 41, 70, 69, 81, 79, 94, 109 and 95. In addition when this compound was eluted, a distinct cucumber odor typical of 2,6-nonadienal was observed. The spectra of the components identified as decatrienal and tridecatetraenal compared well with those of

the C12:3 and C15:4 *n*-aldehydes respectively. The exact structure however remains tentative due to the absence of standards for these two compounds.

Methyl Esters

Methyl esters identified are: methyl propanoate; methyl 4-hexenoate; methyl nonadienoate; methyl dodecatrienoate; methyl pentadecatetraenoate; methyl hexadecatetraenoate. On Carbowax, normal aldehydes and methyl esters of equal chain length had similar retention times, both appearing in one trap. On the SE-30 column the aldehyde eluted before the ester. Propanal and methyl propanoate, hexenal and methyl hexenoate, nonadienal and methyl nonadienoate, and dodecatrienal and methyl dodecatrienoate followed this generalization. The mass spectrum of the peak identified as the C6:1 methyl ester was identical to that of methyl 4-hexenoate published by Rohwedder et al. (13).

Figure 2 shows the spectrum of the compound identi-

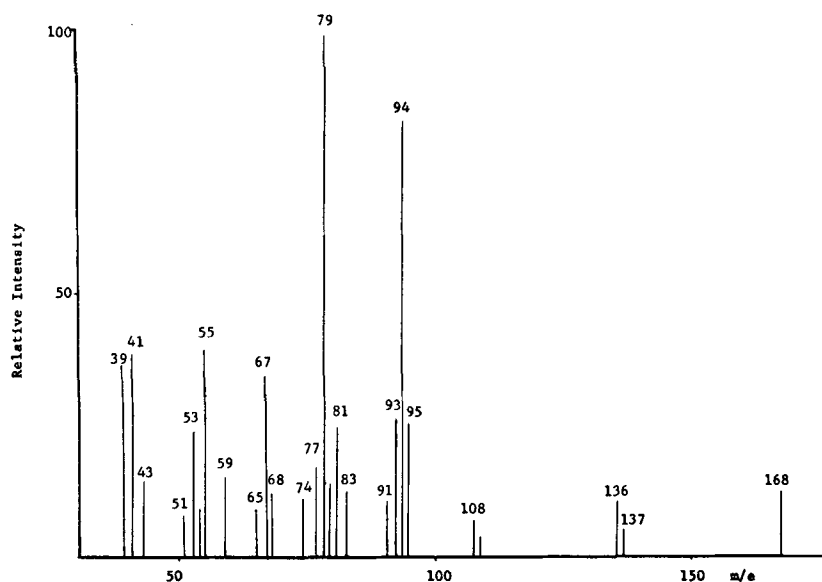


FIG. 2. Mass spectrum of compound identified as methyl nonadienoate.

fied as methyl nonadienoate. Peaks at M-31 and M-32 are characteristic of methyl esters and correspond to the loss of a methoxy group and methanol respectively. A metastable peak at m/e 110.1 indicated the loss of methanol from the molecular ion ($168^+ \rightarrow 136^+ + 32$). The McLafferty rearrangement ion at m/e 74 is small here, while the fragment at m/e 94 (M-74) is greater than 90% of the base peak. This was also the case in the spectra of the nonconjugated dienoates reported by Creveling and Jennings (11), where an extremely intense ion was formed by the loss of the rearrangement fragment (M-74 for methyl esters and M-88 for ethyl esters) at the expense of the rearrangement ion. A metastable peak is noted at m/e 66.4 which indicates one of the modes of production of the base peak at m/e 79 ($94^+ \rightarrow 79^+ + 15$). The alkyl pattern in the lower fragment range observed here (m/e 55, 67, 79, 93) is also seen for ethyl 5,8-tetradecadienoate (11) and in methyl linoleate.

IR analysis of the compound identified as methyl dodecatrienoate showed absorption at 1740 cm^{-1} , 1430 cm^{-1} and 1175 cm^{-1} as typical of esters. The mass spectrum of this compound had a typical methyl ester pattern: M-31, M-32, M-74 and ions at m/e 59, m/e 74 and m/e 87. The hydrocarbon fragments reflect the highly unsaturated nature of the chain and are typical of those noted in the spectra of trienoates reported by Creveling and Jennings (11).

Hydrocarbons

In general the nonoxygenated compounds were much smaller in number and in quantity than the oxygen containing compounds. Both straight chain and cyclic compounds were present. Of the three aliphatic hydrocarbons identified (*n*-octadiene, *n*-undecatriene, *n*-tetradecatetraene), only octadiene was confirmed by comparison of its GC and mass spectrometric (MS) characteristics with the authentic compound. The spectra of the C11:3 and C14:4 hydrocarbons were similar to those of trienes and tetraenes identified previously in irradiated fats (14).

DISCUSSION

Based on the compounds identified in the present study, it is suggested that autoxidation of nonconjugated highly unsaturated fatty acids occurs essentially in accord with the mechanism described by Farmer et al. (15) which involves hydrogen abstraction from alpha methylenic sites, resonance of the resulting radicals and formation of isomeric hydroperoxides. Cleavage of the hydroperoxides results in the formation of a number of decomposition products.

In the case of methyl 4,7,10,13,16,19-docosahexaenoate, $\text{CH}_3\text{-CH}_2\text{-CH=CH-CH}_2\text{-CH=CH-CH}_2\text{-CH=CH-CH}_2\text{-CH=CH-CH}_2\text{-CH=CH-CH}_2\text{-CH}_2\text{-C(=O)OCH}_3$,

where five pentadiene units exist, ten isomeric conjugated monohydroperoxides can be formed. These are the 4-, 7-, 8-, 10-, 11-, 13-, 14-, 16-, 17- and 20-hydroperoxy methyl esters. In addition five nonconjugated hydroperoxidic isomers can be formed with the hydroperoxy group on the 6-, 9-, 12-, 15- or 18- carbon. Formation of the latter group however appears to be much less energetically favored.

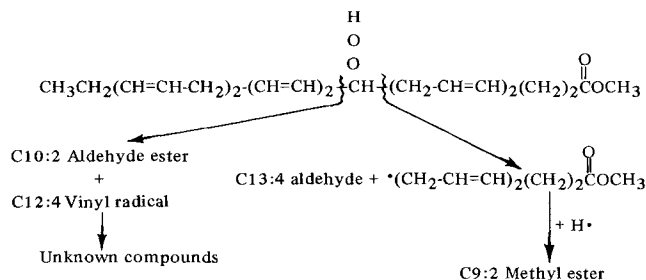
The conjugated hydroperoxides may be divided in two classes depending on the location of the conjugated linkages with respect to the hydroperoxide group:

(A) *Hydroperoxides with the conjugation on the acid side of the hydroperoxide group* (of the general type

$\text{R-C=C-C=C-C=C-C}\cdots\cdots\text{C(=O)OCH}_3$): cleavage of such

compounds produces aliphatic aldehydes, aldehyde esters and hydrocarbons. In case of the 8-hydroperoxy methyl ester, for example, cleavage at the 8,9 carbon-carbon bond would yield a tetraunsaturated C14 alkyl free radical and the C8:2 aldehyde ester. The C14:4 free radical can abstract a hydrogen from another molecule to form the unsaturated hydrocarbon.

Cleavage on the other side of the hydroperoxide group (the 7-8 bond) produces the C15:4 aldehyde and a C7:2 ester radical. The vinyl free radical is unstable and probably polymerizes or decomposes.



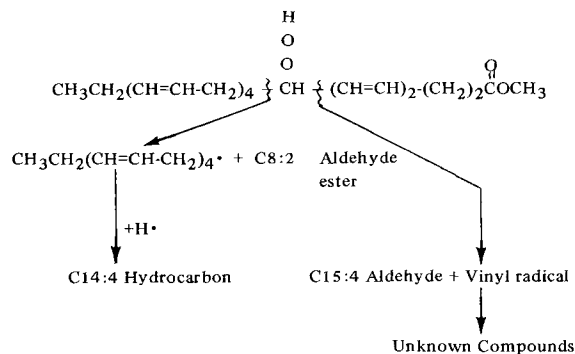
Mechanistic interpretations similar to those discussed above were proposed by Horvat et al. (16) for the autoxidation of 6,9-octadecadiene.

Other hydroperoxides of the (A) structure essentially follow the same pattern of decomposition. Therefore the C12:3 aldehyde arises from cleavage at the 10-11 bond in the 11-hydroperoxide while scission at the other side of the hydroperoxide produces undecatriene. The 14-hydroperoxide gives nonadienal upon cleavage of the 13-14 bond. The unsaturated hydrocarbon (octadiene) would result from scission at the 14-15 bond. With the hydroperoxide on the 17th carbon, scission at the 16-17 bond produces 3-hexenal. Similarly propanal would be formed upon cleavage at the 19-20 bond of the 20-hydroperoxide.

(B) *Hydroperoxides with the conjugation on the hydrocarbon side of the hydroperoxide group* (of the general

type $\text{R-C=C-C=C-C-C-C=C}\cdots\cdots\text{C(=O)OCH}_3$): these intermediates yield upon cleavage methyl esters, aliphatic aldehydes, and aldehyde esters.

In the 10-hydroperoxide methyl ester, cleavage at the 9-10 bond produces C13:4 aldehyde and a C9:2 methyl ester radical. Upon abstraction of hydrogen from another molecule the C9:2 methyl ester is formed. Cleavage at the 10-11 bond yields the C10:2 aldehyde ester and a hydrocarbon vinyl type free radical which probably undergoes polymerization.



Similarly the 4-hydroperoxide produces the C4:0 aldehyde ester by cleavage at the 4-5 bond. Scission at the 3-4 bond would give the C3:0 methyl ester and a C19:6 aldehyde. The latter compound however could not be detected probably because it has too long a retention time on the columns used in this study. In case of the 7-hydroperoxide, scission at the 7-8 bond produces the

C7:1 aldehyde ester and a vinyl type hydrocarbon radical, and cleavage at the 6-7 bond results in the formation of methyl 4-hexenoate. The C13:3 aldehyde ester results from cleavage at the 13-14 bond of the 13-hydroperoxide, while scission on the acid side produces the C10:3 aldehyde and an ester radical which forms methyl dodecatrienoate. By the same mechanism cleavage at the 15-16 bond of the 16-hydroperoxide forms 2,4-heptadienal and the C15:4 methyl ester.

These mechanisms are also in accord with those proposed by Horvat et al. for the diene systems (16).

Among the volatile components identified in the present study, only one compound can be explained on the basis of the decomposition of nonconjugated hydroperoxides. The C6:1 aldehyde ester could result from scission at the 6-7 bond of the 6-hydroperoxide. However decomposition of the 6-hydroperoxy ester would be expected to yield, in addition to vinyl radicals and the 6:1 aldehyde ester, a C17:5 aldehyde which could not be detected under the experimental conditions of this study.

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