Pentane from Thermal Decomposition of Lipoxidase-Derived Products

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ABSTRACT

Thermal decomposition of 13-hydroperoxyoctadeca-9,11-dienoic acid yields hydrocarbons as part of the scission products. Pentane is formed predominately and to the practical exclusion of all other short-chain hydrocarbons.

INTRODUCTION

HYDROCARBONS ARE KNOWN to appear in the early stages of autoxidation of edible fats (1-4). The development of a highly selective and sensitive chromatographic method that allows exclusive determination of hydrocarbons in the presence of volatile polar oxidation products has provided a rapid means of determining the extent of autoxidation (5,6). Hydroperoxides are heat-labile and must undergo some decomposition before derived hydrocarbons are present in the autoxidizing system. Rapid and apparently complete decomposition of the fatty hydroperoxides is achieved by heating the autoxidized mixture to at least 200C. It has been proposed that hydrocarbon formation occurs through splitting of the fatty chain on the alkyl side of the carbon atom containing the hydroperoxide group (1). Other investigators have proposed various splits and free radical rearrangements that yield hydrocarbons among the various autoxidation products (4,7,8).

The exclusive formation of 13-hydroperoxyoctadeca-9,11-dienoic acid by lipoxidase from linoleic acid (9,10) permits compositional studies to be made on the thermal decomposition of a pure hydroperoxide of known structure. Experiments are reported on the formation of hydrocarbons which result from the decomposition of lipoxidase-oxidation products.

EXPERIMENTAL SECTION

The 13-hydroperoxyoctadeca-9,11-dienoic acid was prepared by incubation of lipoxidase with pure linoleic acid as described in previous publications by Dolev et al. (9). The lipoxidase oxidation products were fractionated on a silicic acid column with a methanolic-benzene system, as described by Frankel et al. (11). As determined iodometrically, the peroxide value of the fractionated hydroperoxide, calculated about 8,060 meq. O_2/kg, and UV spectroscopy showed 100% diene conjugation (K_{234} 78.2). The peroxide value reported is above the theoretical value, but, in determining high peroxide values on small samples, large errors are unavoidable.

An autoxidized sample of methyl linoleate (99.8% pure) was used for comparison with the lipoxidized-prepared sample. Autoxidation of pure methyl linoleate (from The Hormel Institute) was conducted at 60C for 27 hr under air with intermittent hand agitation. The peroxide value (iodometric) of this preparation was 362 meq O_2/kg.

Gas chromatographic analyses to determine the hydrocarbon breakdown products of the various fractions were conducted according to methods described by Hoffmann et al. (12) and List et al. (13) with an Al_2O_3 column, which allows passage of the hydrocarbons and irreversibly adsorbs the polar oxygenated materials.

No aliphatic hydrocarbon solvents were used either to extract or fractionate the oxidation products. All solvents were evaporated at low temperatures under a stream of nitrogen. Gas chromatographic control analyses for hydrocarbons were run on all solvents. Diethyl ether was used for extraction; methanol and benzene, for liquid fractionation; and carbon disulfide, for gas chromatographic solutions. Trace amounts of benzene were detected and must be considered in the interpretation of chomatograms.

RESULTS AND DISCUSSION

Fractionation (Fig. 1) of the diethyl ether-soluble lipoxidase-oxidized products from the incubation with linoleic acid showed that 31% of the acid (I) was unreacted and 36% was recovered as pure hydroperoxide (II) plus 25% as a polar-polymeric fraction (IV). An intermediate fraction of 7.3% (III) was discarded. Separation of hydrocarbons by the selective Al_2O_3 gas chromatographic columns is shown

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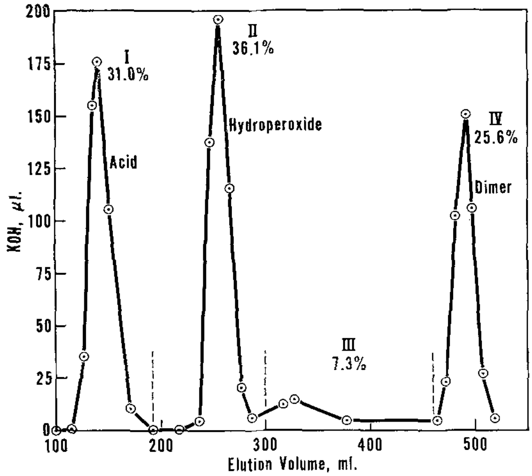


FIG. 1. Separation of lipoxidase-oxidized products of linoleic acid by liquid chromatography on silicic acid. The dimer fraction is assumed to contain small amounts of trimers and higher polymers and polar products.

in Fig. 2A for thermolysis of pure acid hydroperoxide and in Fig. 2B for thermolysis of autoxidized methyl linoleate. To ensure rapid and complete breakdown of hydroperoxides the gas chromatographic injection port, arranged as part of the column extending through the injection heater up to the septum, was operated at 260C. Injecting was done directly into the column.

Pentane is the major volatile hydrocarbon derived from either autoxidized or lipoxidase-derived hydroperoxides. Cleavage of the fatty acid chain on either side of the carbon atom containing the peroxy group results in the formation of a pentane free radical or hexanal (1,2). Hydrogen abstraction by the resulting free radical forms pentane, as shown by the following simplified mechanism:

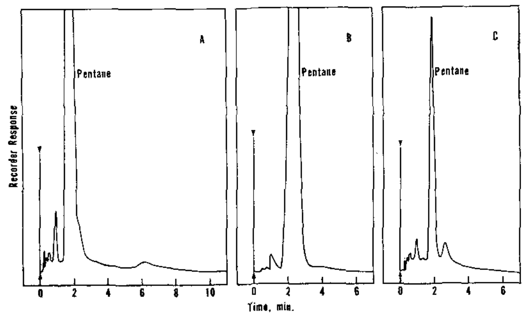
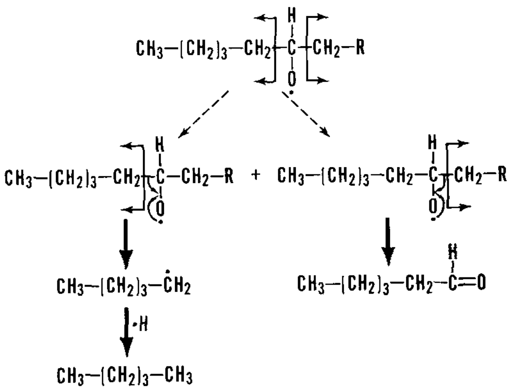
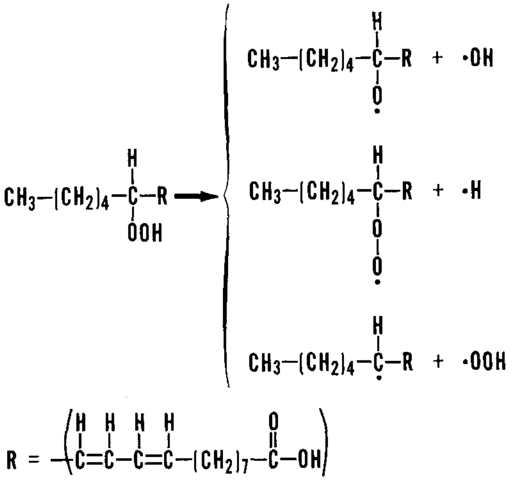


FIG. 2. Hydrocarbons from thermolysis of (A) 13-hydroperoxyoctadeca-9,11-dienoic acid, (B) autoxidized methyl linoleate, and (C) oxidative dimers formed by lipoxidase oxidation of linoleic acid.

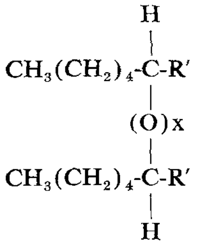


Minor amounts totaling 5% or less of butane, butene, and pentene are indicated in the chromatogram of the lipoxidase-derived product whereas, in the sample from autoxidized linoleate, these components are so small that they are barely indicated. Pentane is also the single major hydrocarbon component derived from the thermal breakdown of the polar-polymeric product which results from lipoxidize oxidation. Fig. 2C, the chromatogram for the polymer, is similar to Fig. 2A for the pure 13-hydroperoxide.

Results indicate that in the oxidative polymer a thermolabile bond exists similar to, and probably derived from, the 13-hydroperoxide linkage. Hydroperoxide decomposition is usually depicted as a free radical breakdown, resulting in formation of one or more of the three following species:



A dimerization between any two of the three species would result in labile products with structure A that would decompose on heating to yield pentane and other products.

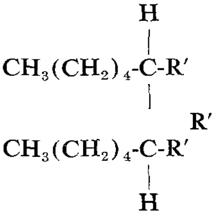


$$\begin{array}{c} \mathrm{H} \\ | \\ \mathrm{CH_3(CH_2)_4\text{-}C\text{-}R'} \\ | \\ (\mathrm{O})x \\ | \\ \mathrm{CH_3(CH_2)_4\text{-}C\text{-}R'} \\ | \\ \mathrm{H} \end{array}$$

$x = 1$ or more

(A)

A structure such as B would be similar to a thermal dimer,



$$\begin{array}{c} \mathrm{H} \\ | \\ \mathrm{CH_3(CH_2)_4\text{-}C\text{-}R'} \\ | \\ \mathrm{CH_3(CH_2)_4\text{-}C\text{-}R'} \\ | \\ \mathrm{H} \end{array}$$

R′ of unknown structure

(B)

would be stable to heat, and could be distilled as methyl esters (14). Mixtures of products with these structures would explain the excess oxygen found in the oxidative dimers (11) as well as the fraction of oxidative dimers reportedly similar or identical to thermal dimers (15). The polymer fraction (IV) showed no conjugated diene absorption, indicating that in polymerization the unsaturation had been modified by free radical addition or cyclization. Unequivocal evidence of the structure(s) of oxidative dimers has never been presented, but these postulations are in accord with current theories of peroxide behavior (7,16-19).

The Al_2O_3 gas chromatographic method, highly selective for removal of oxygenated polar materials, is not sufficiently sophisticated because of temperature limitations to determine quantitatively nonane or any of the higher hydrocarbons. Higher-molecular-weight unsaturated hydrocarbons, above C_8 and including monosubstituted acetylenes (above C_5), would not be detected under present operational limits.

Scission of the fatty acid chain between the 13 and 14 carbon atoms of 13-hydroperoxyoctadeca-9,11dienoic acid is well substantiated by these results, which show that pentane is the predominant short-chain hydrocarbon to arise through thermal decomposition. Pentane need not arise from hexanal as a secondary product of autoxidation, as postulated by Buttery et al. (20). Only minimum amounts of pentane are detected unless the oil is heated to temperatures above the decomposition point of the hydroperoxide, and no increase in the amount of the pentane is observed when 0.025% hexanal is added to an oil.

Preliminary work on autoxidized linolenic acid shows that it yields ethane as the major thermolysis product. When derived from hydrogenated fats, the fatty acid hydroperoxides have different-length, saturated, terminal hydrocarbon chains. These hydroperoxides heat-degrade to yield normal hydrocarbons which contain one less carbon atom than was present in the terminal chain of the hydrogenated fatty acid. Hydroperoxides with an unsaturation terminal to the peroxy group should yield unsaturated hydrocarbons. Formation of specific hydrocarbons by thermolysis of oxidized fatty acids and oxidative polymers provides additional information about their composition and structure.

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