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Volatile Compounds From Thermally Oxidized Methyl Oleate[1]

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ABSTRACT

Thermal oxidation of methyl oleate was studied over a range of temperatures from 50 C to 150 C for periods of time up to 30 min. Degradation was quantitatively followed by gas liquid chromatography (GLC) and liquid scintillation counting of the products of methyl oleate-U-^{14}C heated under a stream of compressed air. Heptane, octane, 2-decanone, benzene, *o*-xylene, methyl hexanoate, methyl heptanoate and methyl octanoate were identified by GLC and mass spectrometry. Mass spectral evidence also was obtained for methyl pimelaldehydate, methyl suberaldehydate and methyl azelaaldehydate. Organic synthesis confirmed the identity of methyl azelaaldehydate. Most of the products formed suggested that autoxidation was responsible for the degradation occurring at the temperatures employed in this study.

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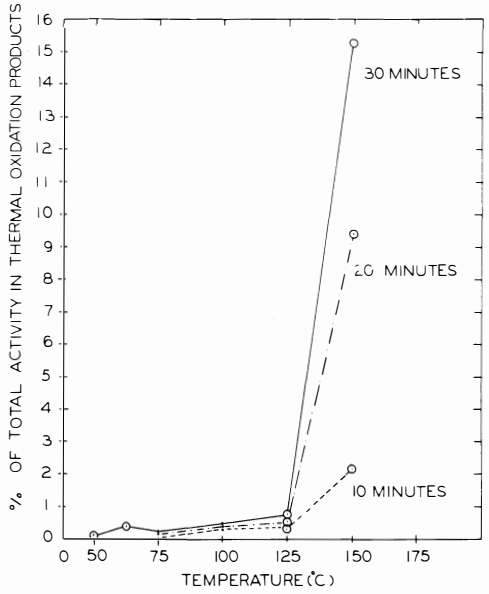


FIG. 1. Per cent of total carbon 14 activity in thermal oxidation products with various time and temperature treatments.

INTRODUCTION

Much of the previous work concerning the degradation of lipids, fatty acids and methyl esters of fatty acids has dealt with autoxidation and its products. The mechanism of autoxidation has been reviewed by several investigators (3,4,10,16), yet no single theory receives total acceptance (8). Carbonyl compounds resulting from the decomposition of the hydroperoxides are important constituents of oxidized fats (4). Flavor and odor thresholds for many of these carbonyls are at levels less than 1 ppm and are important to the sensory qualities of numerous foods (14). Volatile decomposition products of methyl oleate resulting from open air heating at 200 C were examined by Toi et al. (17). Their results showed the presence of C_8 and C_9 aldehydes, semialdehyde methyl esters, C_7 and C_8 hydrocarbons, methyl esters of fatty acids, several fatty acids, mono-methyl esters of dibasic acids and some alcohols.

The objective of this investigation was to evaluate quantitatively and qualitatively the thermal oxidation of methyl oleate heated at various temperatures. Experimental conditions were less extreme than those previously reported by Toi et al. (17), and gas chromatography coupled with mass spectrometry was employed.

EXPERIMENTAL PROCEDURE

Thermal Oxidation of Methyl Oleate

A glass system included a 10 ml Bantamware (Kontes) pear-shaped flask connected to a Bantamware reflux condenser and drying tube. A 1/16 in. Teflon tube was attached to a glass capillary tube that extended to the bottom of the reaction flask. The Teflon tube was connected to a tank of compressed breathing air that purged the sample at a rate of 30 ml/min.

Samples were prepared by adding 10 μl of methyl oleate-U-^{14}C (uniformly labeled), prepared by the procedure of Metcalfe and Schmitz (12) from oleic acid U-^{14}C (sp. act. 630 mc/mM, 99+% radio purity) to 250 μl of unlabeled methyl oleate (99+% purity). Chemicals for this aspect of the study were obtained

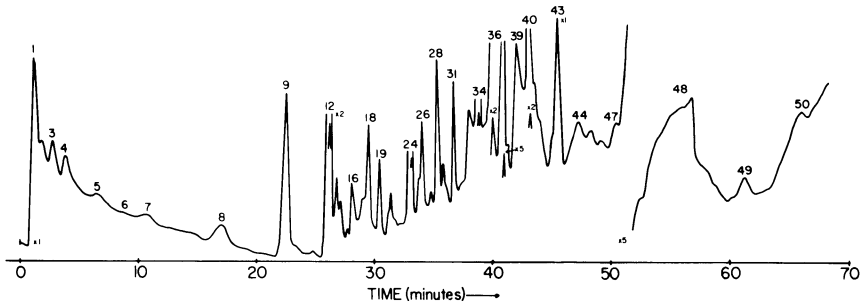


FIG. 2. Gas chromatogram of the volatile compounds from thermally oxidized methyl oleate.

from Applied Science Laboratories. After preparation each sample was subjected to one of the following heat treatments (±0.01 C): 50 C for 30 min; 62 C for 30 min; 75 C for 10, 20 or 30 min; 100 C for 10, 20 or 30 min; 125 C for 10, 20 or 30 min; or 150 C for 10, 20 or 30 min. After heat treatment the samples were stored in tightly-stoppered vials at 5 C until analyzed by gas liquid chromatography (GLC) and liquid scintillation counting.

Samples were analyzed with a modified Aerograph Model 90-P3 gas chromatograph that employed dual 12 ft x 1/8 in. OD stainless steel columns packed with 5% DEGS (diethylene glycol succinate) on Chromosorb G, a Carle Model 100 ultramicro bead detector system, and a Micro-Tek Model GC-2500 linear temperature programmer. These modifications improved the sensitivity of the GLC analysis while allowing the total effluent to be trapped by a Packard Tri-Carb Model 830 gas chromatograph fraction collector modified for subambient temperature operation. Glass cartridges filled with anthracene crystals coated with silicone oil, described by Karmen et al. (7), were used for trapping. Duplicate counts of the trapped fractions were obtained with a Nuclear-Chicago Model 6766 liquid scintillation counter. Each sample was counted to a 1% relative standard deviation (18). The activity of each fraction was calculated as a percentage of the total activity trapped in all fractions of a particular GLC analysis.

Identification of Volatile Compounds

Unlabeled methyl oleate heated at 150 C for 90 min, in the system described above, was used for product identification. The extended heat treatment increased the concentration of components of interest that appeared in the labeled compound experiments. Volatile compounds were analyzed by a combined GLC-mass spectrometer system. An F&M Model 810 gas chromatograph was fitted with a 300 ft x

TABLE I

GLC[a] and Mass Spectral Identification of Some Compounds of Thermally Oxidized Methyl Oleate

Compound	Peak no.	Relative retention time[b]		Mass spectra ref.
		Heated methyl oleate	Authentic	
Heptane	3	0.049	0.050	1
Octane	4	0.065	0.061	1
Benzene	5	0.115	0.117	1
Methyl hexanoate	6	0.362	0.358	2
o-Xylene	---[c]	0.372	0.343	1
Methyl heptanoate	7	0.593	0.595	2
Methyl octanoate	9	1.000	1.000	2
2-Decanone	24	1.406	1.400	2
Methyl pimelaldehydate	43	2.049	---	---
Methyl suberaldehydate	47	2.230	---	---
Methyl azelaaldehydate	48	2.412	2.434	19

[a]GLC, gas liquid chromatography.

[b]Relative to methyl octanoate. 12 ft x 1/8 in. OD stainless steel column packed with 5% DEGS on Chromosorb G. Temperature programmed at 4 C/min from 90-190 C with 15 min initial hold.

[c]Gas chromatographic peak not observed.

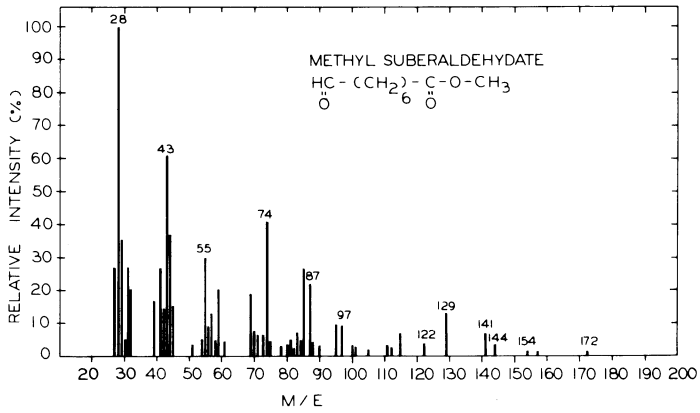


FIG. 3. Mass spectrum of compound tentatively identified as methyl suberaldehydate.

0.01 in. ID Golay type capillary column with butanediol succinate (BDS) as the stationary liquid phase. Operating conditions for this inlet system were 180 C isothermal column temperature with a flow rate of 1 ml per min of helium.

The Golay column was directly connected to the double ion source of an Atlas CH-4 mass spectrometer; the high vacuum was 1.5 x 10^{-6} mm of Hg while admitting GLC effluent. The double ion source gave both mass spectral oscillograms and a strip chart gas chromatogram.

GLC effluent fractions were also collected from a 12 ft x 1/8 in. OD stainless steel column containing 5% DEGS on Chromosorb G, rechromatographed and recollected from a 12 ft x 1/8 in. OD stainless steel column containing 3% BDS on Chromosorb G, and examined with the static reservoir inlet system of the Atlas CH-4. This technique enabled low voltage studies and also allowed the accurate measurement of the parent $(P)^+$, $(P+1)^+$ and $(P+2)^+$ ion intensities which are often helpful in assigning an empirical formula.

A third mass spectrometer inlet system utilized the EC-1 gas inlet valve which permitted regulation of the amount of GLC packed column effluent admitted to the double ion source of the Atlas CH-4. The column used with this inlet system was a 12 ft x 1/8 in. OD stainless steel column containing 5% DEGS on Chromosorb G. Operating conditions were a flow rate of 25 ml of helium per min and a temperature program from 90-190 C at 4 C per min after a 15 min initial hold.

Methyl azelaaldehydate was synthesized from methyl oleate via hydroxylation with osmium tetroxide and subsequent oxidative cleavage with lead tetra-acetate. This synthesis yielded *n*-nonanal, nonanoic acid, and methyl azelaaldehydate which were easily separated by GLC and analyzed with the static reservoir inlet system of the Atlas CH-4. In retrospect the synthesis of methyl azelaaldehydate via ozonolysis and subsequent purification as the bisulfate compound appears to be a better route

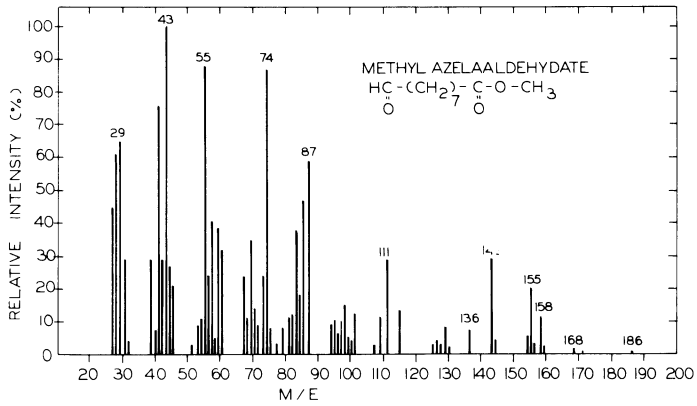


FIG. 4. Mass spectrum of authentic methyl azelaaldehydate.

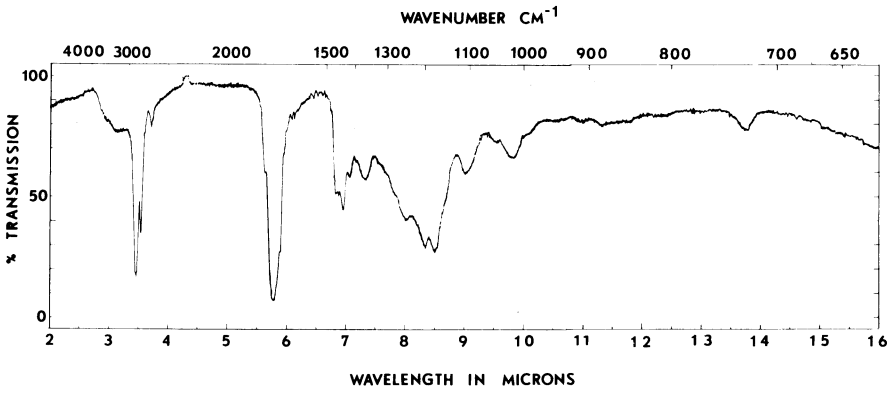


FIG. 5. IR spectrum of authentic methyl azelaaldehydate.

(13).

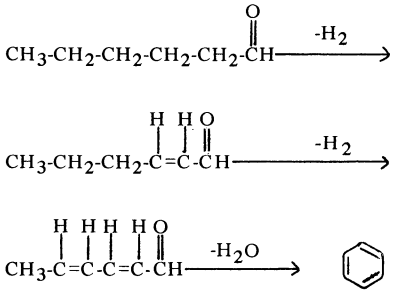
IR analyses were performed with a Beckman IR-5A spectrometer.

RESULTS AND DISCUSSION

Quantitative data in Figure 1 show the percentage of the total carbon 14 activity that appeared in GLC fractions other than the methyl oleate fraction. Of the total sample carbon 14 activity in the original sample, approximately 13% appeared in all volatile fractions including methyl oleate and the remaining 87% apparently remained in the polymeric fraction or free acid form. Thermal oxidation products accounted for less than 1% of the total activity through the 125 C treatments.

Significant production of oxidation products did not occur until the temperature of the reaction reached 150 C. The temperature of the treatment is a more important factor than time in the thermal oxidation process.

The gas chromatogram of the 150 C sample (Fig. 2) shows the presence of approximately 50 components from the thermally oxidized methyl oleate. Evidence for an additional 12 compounds was obtained in related chromatographic analyses. Some of the major volatile compounds were identified (Table I). The presence of the C_7 and C_8 *n*-alkanes has been reported in gamma-irradiated milk fat (9), gamma-irradiated methyl oleate (11), and in methyl oleate held at 200 C (17). These compounds appeared abundantly in the low (50 C, 30 min) and high (150 C, 30 min) temperature-treated systems of this study. Benzene was present in much smaller quantities than the *n*-alkanes and may be an artifact. Its presence was reported in gamma-irradiated milk fat (9) and in gamma-irradiated methyl oleate (11). Khatri (9) has indicated that aldehydes of six carbons or more tend to cyclize to aromatic compounds at high temperatures in the inlet system of the mass spectrometer. The aldehyde may possibly undergo a dehydrogenation and a subsequent dehydration reaction similar to the one shown below at elevated temperatures found in the inlet system of the mass spectrometer as well as in the model system reactions.



Fritsch and Deatherage (6) found the C_6, C_7 and C_8 saturated fatty acids as decomposition products of methyl oleate. Subsequently Toi et al. (17) found the C_6, C_7 and C_8 fatty acid methyl esters from heated methyl oleate. The presence of 2-decanone has previously been reported by Scanlan et al. (15) in heated milk and was present in the heated methyl oleate in limited concentrations relative to the amount of short chain fatty acids present. It has been well established that the odd numbered *n*-alkyl methyl ketones are produced from beta-keto acids; however the precursors and mechanisms of formation of the even-numbered *n*-methyl ketone have not been elucidated. The presence of the 2-decanone in this system suggests that an ester of oleic acid may serve as a precursor for its formation.

The semialdehyde methyl esters have been studied previously (4,5,17), but spectrometric data were available for only methyl azelaaldehydate (19). The mass spectrum of the fraction

tentatively identified as methyl suberaldehydate was obtained using the 70 ev ion source and the static reservoir system (Fig. 3).

Low voltage spectra at 11 ev helped establish the apparent molecular ion at m/e 172 and 0.6% relative intensity to the base peak. Peaks at m/e 74 (41%) and 87 (22%) were indicative of a methyl ester. The base peak at 28 (100%) along with the peak at 144 (4%), P-28, may result from the carbonyl moiety. Other characteristic peaks were 129 (12%), 141 (7%), 115 (7%) and 154 (1%). The mass spectrum of the fraction tentatively identified as methyl azelaaldehydate showed an apparent molecular ion at m/e 186 (0.01%) and a base peak at 28 (100%) with the associated peak at 158 (8%), P-28. Peaks at m/e 74 (88%) and 87 (63%) were again indicative of a methyl ester. Other characteristic peaks that were related to the methyl suberaldehydate by the mass difference of one methylene unit were 143 (21%), 155 (16%) and 129 (14%).

The mass spectrum of authentic methyl azelaaldehydate shown in Figure 4 is in agreement with the spectrum of the tentatively identified compound and also with the spectrum of authentic methyl azelaaldehydate obtained from the Northern Regional Research Laboratory, Peoria, Illinois. However the mass spectrum of methyl azelaaldehydate recently published by Yasuda et al. (19) is somewhat different. Certain ions, m/e 168 (P-18), 158 (P-28) and 155 (P-31) which we observed and found important in mass spectral interpretation, are absent in this previously published spectrum (19).

Figure 5 is the infrared spectrum of authentic methyl azelaaldehydate. There are strong CH stretch bands at 2700 and 2900 cm^{-1} and a strong aldehyde carbonyl stretch at 1740 cm^{-1}.

The infrared spectra of methyl suberaldehydate and methyl azelaaldehydate from thermally oxidized methyl oleate showed characteristic fingerprint bands similar to the authentic methyl azelaaldehydate. Mass spectral evidence also suggested the presence of the next lower member of the homologous series, methyl pimelaldehydate. A linear relationship was observed in a plot of relative retention times versus carbon number for the three semialdehyde homologs. The observed odor of the semialdehyde fractions was suggestive of the objectionable oxidized odor of cooking fats.

In conclusion at least 62 compounds were separated from heated methyl oleate. Mass spectral evidence has been given for several volatile products, the major component being methyl azelaaldehydate. Two other members of the homologous series of semialdehyde methyl esters were tentatively identified and each appeared to have important sensory qualities in heated fats.

ACKNOWLEDGMENTS

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