Identification of Some Acids From Autoxidation of Methyl Linoleate

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Abstract

The acids from autoxidation of methyl linoleate have been analyzed as their methyl esters by combined capillary gas chromatography-mass spectrometry (GC-MS). The principal components were hexanoic, trans-2-octenoic, suberic and azelaic acid. Minor components included formic, pentanoic, heptanoic, trans-2-heptenoic, octanoic and nonanoic acid. In addition, *trans*-2,3-epoxyoctanoic acid was isolated as its methyl ester by preparative GLC and was identified by means of NMR, high resolution MS, IR and by conversion to a known derivative.

Introduction

Several authors have reported mid-length fatty acids in oxidized polyunsaturated vegetable oils. Chang and co-workers (1,2), for example, have identified a series of fatty acids from thermal oxidation of corn oil and from mildly autoxidized soybean oil. Toi et al. (3) identified several acids from thermal oxidation of purified methyl oleate. Our work extends the information to include products from methyl linoleate. The identification of several acids from autoxidation of methyl linoleate are reported, together with some possible mechanisms of their formation.

Experimental Procedures

Oxidation of Methyl Linoleate

Seventy-five grams of methyl linoleate (97–99% pure as analyzed by GLC, peroxide number <1 mmole/kg) was oxidized for two weeks as a film on purified glass wool continuously exposed to purified oxygen (793 mm mercury) at room temperature in laboratory light. After oxidation the ester had a peroxide number of approximately 1000 mmole/kg. The methyl linoleate was oxidized to a PV of 1000 because only limited quantities of pure ester (97–99% as analyzed by GLC) were available at this time and our previous experience had shown that autoxidation of small quantities (20–30 g) of ester to low peroxide values did not provide a sufficient quantity of volatile material for GC-MS analysis. Another difficulty observed, was the inability to stop the reaction at an intermediate peroxide level once acceleration of oxygen uptake occurred (after 4–6 days). Peroxide value can increase several fold in a 4–8 hr period during this accelerated period. The results from this experiment may not relate to oxidative off-flavor development in oils which are observed at low peroxide values.

Preparation of Methyl Esters

After vacuum transfer of the volatiles ($2–4 \times 10^{-2}$ torr), the autoxidized ester residue was dissolved in 1 liter of ether and the ether solution was extracted with three 200 ml portions of saturated sodium bicarbonate solution. Hydrochloric acid (0.1 M) was added with rapid stirring to the bicarbonate solution contained in an ice bath to a pH of about 7 (pH paper). Bicarbonate was used instead of the stronger base carbonate, even though the latter would be more efficient for extracting long chain acids from the oxidized ester to minimize artifact formation (weak bases can catalyze aldol condensations of aldehydes and addition of nucleophilic agents to double bonds in α,β-unsaturated acids). The use of bicarbonate for extracting acids could result in incomplete removal of long chain acids and thus our results are probably not quantitative. Also, it is possible that other acids were present which were not extracted and thus not identified by our analytical techniques. The liberated acids were extracted with three 200 ml portions of ether. The ether solution was dried over anhydrous magnesium sulfate, and reduced to about 50 ml in a rotary evaporator at 20–30 torr. A slight excess of diazomethane (persistence of yellow color) was added to the ether concentrate at 0 C, and the excess ether and diazomethane were removed at reduced pressure (20–30 torr). The methyl ester fraction thus obtained had a volume of 2.4 ml.

GC-MS Analysis

One –0.5 μliter of the ester fraction was analyzed on a 200 ft, 0.01 in. capillary column coated with General Electric SF 96–50 silicone oil, 1% Carbowax 20M. The GC-MS technique with the Bendix Time-of-Flight spectrometer has been described in prior publications (4,5).

High resolution mass spectral analyses were obtained with a CEC 21–110 B double-focusing mass spectrometer. Because nitrogen, sulfur and other hetero-atoms were known to be absent, the oxygen contents of given mass peaks were easily established by comparing them with isobaric peaks of known oxygen content from standard compounds.

Results and Discussion

The chromatogram shown in Figure 1 is typical of those from several replicate samples. Table I lists

TABLE I

Acids Identified as Their Methyl Esters From Autoxidized Methyl Linoleate by Combined Capillary GC-MS

Peak number[a]	Acids
1	Formic
2	....
3	Pentanoic
4	Hexanoic
5	Heptanoic
6	*Trans*-2-heptenoic
7	*Cis*-2-octenoic[b]
8	Octanoic
9	*Trans*-2-octenoic
10	Nonanoic
11	Not identified
12	Not identified
13	*Trans*-2,3epoxyoctanoic
14	Not identified
15	Not identified
16	Not identified
17	Not identified
18	Suberic acid
19	Azelaic acid

[a] Refer to chromatogram of Figure 1. Some small peaks have not been numbered.

[b] This compound is only tentatively identified since no authentic sample of *cis*-2-octenoic acid was available.

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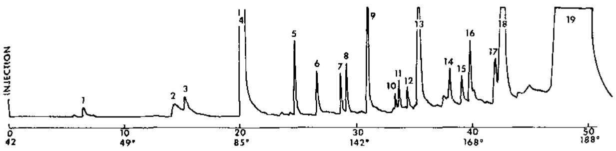


FIG. 1. Gas chromatogram of methyl esters from oxidized methyl linoleate.

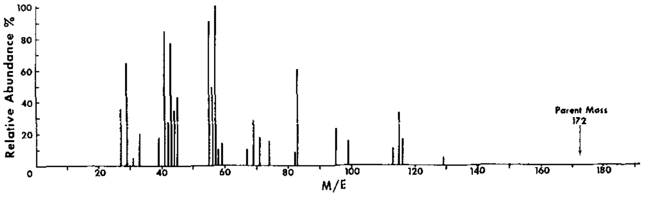


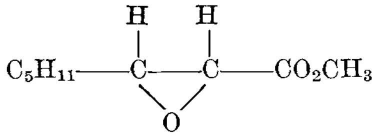
FIG. 2. Mass spectrum of methyl 2,3-epoxy-octanoate.

the acids identified according to peaks as numbered in Figure 1. The mass spectral data were compared with those of authentic compounds or with literature data; in addition, all compounds were confirmed by GLC retention times. The list contains several acids and a 2,3-epoxy acid not previously reported. The peaks numbered 11, 12, and 14–17 have not been identified. It appears that carbon dioxide and pentane present in peak 1 and hexanal, peak 2, are artifacts (Fig. 1). Also, the peak area, in the GLC chromatogram, of methyl formate should not be used as an estimate of its concentration since loss of methyl formate probably occurred during concentration of the ester fraction. Again, formic acid and other low molecular weight acids might have been lost during vacuum distillation of the oxidized ester prior to extraction with bicarbonate.

The mass spectrum obtained for peak 13 (Fig. 2) during the GC-MS analysis did not give sufficient data for a structure suggestion. A small quantity of this peak was collected from a gas chromatograph equipped with a 4 ft × ¼ in. column packed with 20% Apiezon L on Chromosorb P. Analysis of this fraction by TLC using 10% ether-petroleum ether for development and using the 200 ft, 0.01 in. capillary column previously described showed only one component.

Examination of this fraction by infrared spectroscopy (Fig. 3) showed bands at 1250 cm^{-1}, 890 cm^{-1} and 790 cm^{-1} (the region of the 8, 11 and 12 μ, so-called epoxide bands) (6), suggesting the presence of an epoxide ring in the ester.

The ion fragment at mass 115 in the mass spectrum was shown to contain one oxygen by comparison with the ion peak due to $CH_3O_2C(CH_2)_4+$ from methyl nonanoate. Similarly, other ion peaks (masses 101, 129, 143, etc.) were shown to contain three oxygens. A very weak parent peak at 172 was obtained. The data suggested the following epoxy ester:



In order to obtain chemical evidence for this proposed structure, a small quantity of the GLC fraction was reduced with sodium borohydride in methanol at 0 C and the resulting β-hydroxy methyl octanoate was identified by mass spectrometry. The infrared spectrum of this compound possessed a strong band at 1735 cm^{-1} and a broad band at 3450 cm^{-1}, indicating the presence of hydroxyl and ester groups, consistent with the proposed structure.

An authentic sample of methyl 2,3-epoxyoctanoate was prepared by condensation of hexanal (freshly prepared, 97% pure as analyzed by GLC) and methyl bromoacetate in dry benzene at 10 C using a sodium hydride catalyst. The compound was isolated from the reaction mixture and purified by preparatory GLC (7). Two fractions were separated on the 4 ft × ¼ in. column previously described. These gave identical mass spectra and were therefore assumed to be geometric isomers. Also, the mass spectra of these two fractions were identical to that of the epoxy ester from autoxidized methyl linoleate. The second GLC fraction possessed the same GLC retention time as the epoxy ester from autoxidized methyl linoleate. Its infrared spectrum was also identical to that of the isolated epoxy ester. The NMR spectrum further aided the confirmation of the identity of the original compound (peak 13) with one of the isomers of the synthetic 2,3-epoxy-octanoate.

The first fraction from the synthetic methyl 2,3-epoxyoctanoate was examined in carbon tetrachloride by NMR at 60 MHz. The alpha epoxy proton gave rise to a sharp doublet ($J = 4.2$ Hz) at $\tau = 6.70$ and the beta proton to a broad incompletely resolved multiplet at $\tau = 7.00$.

On the basis of a comparison of published J values (5.5 Hz) for several substituted *cis* epoxy esters (8), the isomer corresponding to the first peak is *cis*-methyl 2,3-epoxyoctanoate. The NMR spectrum of the second GLC fraction revealed a sharp peak at 6.95 τ corresponding to two protons, plus a broad peak at 6.97 τ, indicating overlap of the two *trans*-hydrogen of the ring [$\tau = 6.9$ for methine hydrogen on the epoxide ring (6)]. The trans configuration was thus assigned to the isomer corresponding to the second GLC peak.

Presumably *trans*-2,3-epoxyoctanoic acid could form by epoxidation of the carbon-carbon double bond in 2-octenal, previously identified from autoxidized methyl linoleate (9,10), followed by subsequent oxidation of 2,3-epoxyoctanal to 2,3-epoxyoctanoic acid. Hacman and Perret have shown that autoxidation of 2-ethyl-2-hexenal proceeds through 2-ethyl-2, 3-epoxyhexanoic acid (11). Brill and co-workers have established that epoxide can readily form from the reaction of peroxy free radicals and certain olefins (12). Thus a second possible pathway for production of 2,3-epoxyoctanal may be the reaction of 2-octenal and peroxy free radicals.

The other acids identified in this study could result from oxidation of the aldehydes formed during autoxidation of methyl linoleate, because it is well known that aldehydes readily oxidize to the corresponding acids. However, it should be noted that all the aldehydes corresponding to these acids have not been identified.

No attempt was made to determine whether azelaic

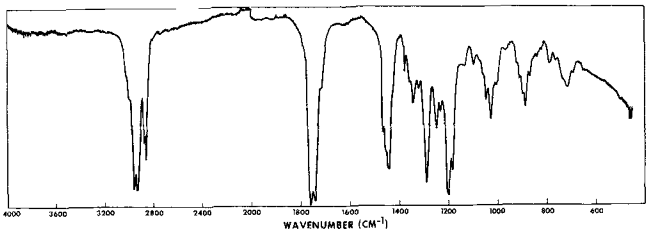


FIG. 3. Infrared spectrum of *trans*-methyl-2,3-epoxyoctanoate.

or suberic acids originally existed as free acids or as half-acid esters. The identification of half-acid esters from autoxidized methyl oleate and oxidized vegetable oils makes it appear reasonable that these two compounds were originally present as half-acid esters.

Several of the principal fatty acids identified in our study were also found in the acid fraction from oxidized corn oil by Chang and co-workers (1,2). They were: pentanoic, hexanoic, 2-octenoic, suberic and azelaic acids. Qualitative differences in the acid fractions from methyl linoleate and corn oil are probably a reflection of the greater complexity of corn oil as compared to methyl linoleate.

Toi and co-workers identified methyl suberate and methyl azelate from thermal oxidation of methyl oleate (3). They suggested that these two major acids were formed from oxidation of semi-aldehydes, which were first formed by thermal decomposition of the 9 and 10-hydroperoxides of oleic acid. The same two esters were identified in the acid fraction from autoxidation of methyl linoleate and could form by a similar mechanism from the 9 hydroperoxide and the hypothetical allylic 8 hydroperoxide.

ACKNOWLEDGMENTS

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