

# Thermal Oxidation of Lipids in Monolayers II. Unsaturated Fatty Acid Esters

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To investigate whether oxidation of lipids in the ordered state differs from that in the bulk phase upon heating, ethyl oleate, ethyl linoleate and ethyl linolenate adsorbed as monolayers on silica were used as model systems. For both bulk and monolayer samples, the major decomposition products found were those typical of classical decomposition of hydroperoxides. However, quantitative and qualitative differences were observed after heating. The decomposition patterns in monolayers were much simpler than those of the bulk samples.

The major decomposition products of the substrates in monolayers were ethyl 9-oxononanoate and nonanal from ethyl oleate, ethyl 9-oxononanoate and hexanal from ethyl linoleate, and ethyl 9-oxononanoate and hexenal from ethyl linolenate. It is likely that the acidic nature of silica favored a selective heterolytic cleavage of hydroperoxide intermediates.

Lipid oxidation has been the subject of intensive research for many years. Most studies have involved the investigation of oxidation rates, products of oxidation and mechanisms of oxidative reactions in bulk lipids. However, in biological systems such as cell membranes the lipid molecules are highly ordered. The question arises whether mechanisms of oxidation in bulk and the resulting products differ from those in the ordered state. In recent years, extensive research has been carried out to study the mechanisms and oxidation products of lipid oxidation with emphasis on biological systems (1). Because the study of ordered lipid molecules as they exist in biological systems is extremely complex, most investigators used simpler model systems, such as silica monolayers (2,3), liposomes (4) or red blood cell ghosts (5), to provide information which may be applicable to the more natural systems. In our laboratory, a study was initiated in which various lipids adsorbed on silica were chosen as the model systems, and the nature of their binding investigated (6). The purpose of the present work was to study thermal oxidation of unsaturated fatty acid ethyl esters adsorbed in monolayers.

## MATERIALS AND METHODS

Ethyl oleate, ethyl linoleate and ethyl linolenate were purchased in the highest available purity from Sigma Chemical Co., St. Louis, Missouri. These were used without further purification.

Silica gel G was purchased from Applied Science Laboratories, State College, Pennsylvania. The material had a particle size of 10–40  $\mu$  and contained about 13% calcium sulfate as binder.

*Silica monolayer preparation.* The lipid monolayer on silica was prepared according to the procedures described previously (7).

*Heat treatment.* One-g sample of substrate, either adsorbed on silica or in bulk, was placed in a 200-ml round bottomed flask fitted with a ground glass stopper and heated in oven at 180°C for one hr.

*Oxidation rate of ethyl linoleate (18:2 EE).* The rate of oxidation for ethyl linoleate was followed by determining the amount of substrate changed. Both monolayer and bulk samples were incubated either at 60°C or 180°C for the desired length of time and the lipid extracted three times with acetone. Recovery of ethyl linoleate from silica gel by acetone was 96–98%. The extract was then analyzed by GLC and the amount of the unchanged substrate calculated from a previously prepared standard curve.

*Epoxidation.* Epoxidation of oleic acid or linoleic acid was carried out by mixing the unsaturated fatty acids with peracetic acid in glacial acetic acid solution as described by Findley and co-workers (8). Peracetic acid was prepared by treating hydrogen peroxide with acetic anhydride without any catalyst. The final epoxide product gave a single spot when analyzed on TLC plates using petroleum ether/diethyl ether/acetic acid (80:20:1, v/v/v).

*Methylation.* Methylation was carried out by mixing ca. 10 mg of thermally oxidized acid epoxide with 200  $\mu$ l of meth-Prep II (Applied Science Lab Inc.). This reagent is a methanolic solution of m-trifluoromethylphenyl trimethylammonium hydroxide which effects a fast reaction at room temperature and requires no extraction or additional treatment prior to GC analysis (9).

*Collection of volatiles.* Volatiles were collected by cold-finger distillation as described by Nawar et al. (10). Each sample was distilled under vacuum for one hr at 80°C. To minimize peak overlap in subsequent GC analysis the distillate was fractionated into polar and nonpolar fractions. The cold-finger was rinsed with 25 ml of pentane, and this fraction of the distillate was added to five g of silica gel which had been activated at 110°C for several hr. The mixture was stirred for 25 min, then filtered using a Buchner funnel with a scintered glass plate. The silica was washed with 10 ml of pentane and the filtrates combined. This filtrate contained the nonpolar compounds. Polar compounds were removed by washing with 25 ml of diethyl ether after rinsing with 100 ml of pentane to remove any traces of nonpolar compounds. Both fractions were slowly concentrated under nitrogen to about 0.1 ml for further analysis.

*Analysis of volatile decomposition products.* The gas chromatograph used was a Perkin Elmer model 3920 B equipped with a flame ionization detector. The temperature was programmed from 60°C to 200°C at 4°/min. A Carbowax 20 M capillary column (500 ft  $\times$  0.02 i.d.) with a carrier gas flow rate of 8 ml/min was

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used. Thermal decomposition products were identified by gas chromatography-mass spectrometry (GC-MS). Heptadecane and octan-3-one were used as internal standards for the quantitation of the major peaks. When appropriate correction factors were used, based on pre-testing with mixtures of available authentic compounds, recoveries higher than 90% were obtained for most of the compounds tested.

## RESULTS AND DISCUSSION

At 60°C ethyl linoleate adsorbed on silica oxidized at a faster rate than that in bulk (Fig. 1). It took four hr for the monolayers and 10 hr for the bulk samples to reach approximately 50% oxidation based on loss of substrate. At 180°C, the rate of oxidation was much faster for both the monolayer and the bulk samples than those heated at 60°C. However, except for the initial stage, oxidation of the bulk samples was faster than that of the monolayers. It took one hr for the monolayer sample and 40 min for the bulk sample to reach approximately 80% oxidation. The faster oxidation of linoleate in monolayers at 60°C is in agreement with the results of other investigators (2, 3), and probably is due to the larger surface and more facile exposure to oxygen in the case of the monolayers. This, however, appears to be offset by the higher mobility of the lipid molecules and the faster radical transfer in bulk at the higher temperature.

Quantitative analyses of the volatile decomposition products from oleate, linoleate and linolenate adsorbed on silica and in bulk, after heating at 180°C for one hr, are given in Tables 1-4. For both the adsorbed and bulk samples, the major volatile products found were

those expected from hydroperoxide decomposition (11). However, major qualitative and quantitative differences were observed.

For all the substrates used, bulk samples produced much more ethyl octanoate than those in the ordered state. The monolayers of ethyl linoleate and ethyl linolenate produced more ethyl 9-oxononanoate than the corresponding bulk samples. However, ethyl 9-oxononanoate produced from ethyl oleate monolayer was slightly less than that from the bulk sample.

Because as shown above, the rate of oxidation varies with both temperature and physical state (i.e., monolayer vs bulk), the volatiles from ethyl linoleate on silica and in bulk were compared at equivalent levels of oxidation. As shown in Table 5, the same trends were observed, i.e., bulk sample produced more ethyl octanoate than that of silica, whereas silica samples produced more ethyl 9-oxononanoate than that of bulk, regardless of temperature, heating period or degree of oxidation. The fact that the same qualitative pattern of volatiles was found at high and low temperatures implies that the same mode of decomposition occurs over the temperature range studied.

Mead and co-workers (2) suggested that epoxides rather than hydroperoxides might be the major intermediates for the oxidation of unsaturated fatty acids adsorbed on silica, presumably because of the proximity of the unsaturated fatty acid chains on the silica surface. To test this hypothesis, the 9,10-epoxyoctadecenoic and 12,13-epoxyoctadecenoic acid mixture from linoleic acid was synthesized and heated at 180°C for one hr. The decomposition products were then methylated and analyzed by GC/MS. The major products were hexanal and 9-oxonanoate (Fig. 2), as in the case of oxidation on silica. When the 9,10-epoxyoctadecenoic acid from oleic acid was treated similarly, nonanal was the major product formed. Furthermore, TLC analysis of the silica oxidation products gave rise to a significant spot corresponding to the synthesized epoxide as observed by Mead's group. When the analysis was repeated after reaction with phosphoric acid, which converts epoxide to diol (12), the original spot was no longer visible. It is clear that epoxides are formed; their formation, however, may have occurred as a result of a catalytic effect, unique to silica rather than as a result of interaction between rigidly oriented neighboring molecules as explained by Mead and his co-workers.

It is quite possible, however, that hydroperoxide intermediates were also major primary products in the case of the adsorbed phase oxidation, and that the acidic nature of silica favored a selective heterolytic cleavage. Kimoto and Gaddis (13) reported that HCl-treated Fuller's earth decomposed autoxidized trilinolein into a greater yield and more selective distribution of carbonyl compounds than those obtained from treatment of the autoxidized trilinolein with a free radical catalyst. They further showed that the volatiles were mainly hexanal and nonenal. Gardner and Plattner (14) reported that treatment of isomeric methyl linoleate hydroperoxides with  $\text{BF}_3$ , a Lewis acid, leads to a carbon-to-oxygen rearrangement that causes cleavage into shorter chain carbonyl compounds. For methyl linoleate hydroperoxides, hexanal, nonanal, methyl 9-oxononanoate and methyl 12-oxododecenoate were produced.

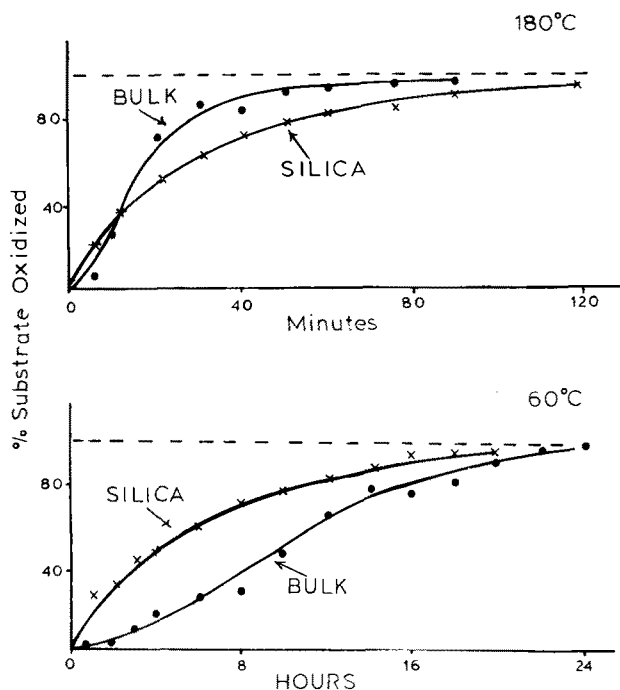


FIG. 1. Rate of ethyl linoleate oxidation on silica and in bulk at 60 and 180°C.

## THERMAL OXIDATION OF LIPIDS IN MONOLAYERS II.

TABLE 1

Quantitative Analysis of The Decomposition Products of Ethyl Oleate Heated at 180°C for 1 Hr (polar fraction)

Peak No.	Compound	Silica ( $\mu\text{mol/g}$ )		Bulk ( $\mu\text{mol/g}$ )	
		Mean <sup>a</sup>	SD <sup>b</sup>	Mean	SD
1	Hexanal	8.2	2.6	9.4	1.4
2	Heptanal	12.0	2.4	13.3	0.4
3	Octanal	16.2	1.8	17.7	0.4
4	Et heptanoate	0.5	0.1	8.1	1.8
5	Nonanal	27.8	3.4	19.2	1.9
6	Et octanoate	0.6	0.1	10.4	2.5
7	2-decenal	9.9	0.7	17.7	2.0
8	2-undecenal	6.3	0.3	9.2	1.2
9	Et 7-oxoheptanoate	2.2	0.3	5.2	0.1
10	Et 8-oxooctanoate	2.7	0.6	6.2	0.7
11	Et 9-oxononanoate	5.2	0.5	6.0	0.8

<sup>a</sup>Mean from 2 determinations.<sup>b</sup>Average deviation from the mean.

TABLE 2

Quantitative Analysis of Decomposition Products of Ethyl Linoleate, Heated at 180°C for 1 Hr (polar fraction)

Compound	Silica ( $\mu\text{mol/g}$ )		Bulk ( $\mu\text{mol/g}$ )	
	Mean <sup>a</sup>	SD <sup>b</sup>	Mean	SD
Hexanal	42.4	5.6	27.7	1.9
Et hexanoate	2.8	0.9	7.3	0.4
Et heptanoate	1.7	0.5	9.4	1.0
Et octanoate	2.0	0.1	15.4	0.9
Et 6-oxo-hexanoate	0.4	0.2	1.5	0.3
c,t-2,4-decadienal	2.2	0.3	3.8	0.3
t,t-2,4-decadienal	4.1	0.3	6.5	0.2
Et 7-oxoheptanoate	2.2	0.5	2.7	0.4
Et 8-oxooctanoate	5.8	0.8	9.2	0.1
Et 9-oxononanoate	22.3	2.3	16.6	0.6

<sup>a</sup>Mean from 2 determinations.<sup>b</sup>Average deviation from the mean.

TABLE 3

Quantitative Analysis of Thermal Decomposition Products of Ethyl Linolenate, Heated at 180°C for 1 Hr (polar fraction)

Compound	Silica ( $\mu\text{mol/g}$ )		Bulk ( $\mu\text{mol/g}$ )	
	Mean <sup>a</sup>	SD <sup>b</sup>	Mean	SD
Pentenal	5.2	0.2	6.6	0.2
Hexenal	6.1	0.3	0.6	0.1
Et hexanoate	trace <sup>c</sup>	-	0.7	0.1
Heptenal	0.7	0.1	1.2	0.1
Et heptanoate	0.4	0.1	1.8	0.2
Et octanoate	3.5	0.2	17.9	1.2
c,t-2,4-heptadienal	3.9	0.3	6.1	0.3
t,t-2,4-heptadienal	9.4	0.2	17.8	0.2
Et 6-oxohexanoate	0.3	0.1	1.1	0.3
Et 7-oxoheptanoate	0.5	0.1	2.2	0.5
Et 8-oxooctanoate	2.5	0.1	6.7	1.4
Et 9-oxononanoate	16.3	0.8	11.9	0.7

<sup>a</sup>Mean from 2 determinations.<sup>b</sup>Average deviation from the mean.<sup>c</sup>Trace; <0.1  $\mu\text{mol/g}$ .

TABLE 4

Major Nonpolar Thermal Decomposition Products Recovered from Ethyl Oleate, Ethyl Linoleate and Ethyl Linolenate After Heating at 180°C 1 Hr

Compound	Silica ( $\mu\text{mol/g}$ )		Bulk ( $\mu\text{mol/g}$ )	
	Mean <sup>a</sup>	SD <sup>b</sup>	Mean	SD
Ethyl oleate				
nonene	0.56	0.09	0.43	0.01
decane	trace <sup>c</sup>	-	0.18	0.05
decene	0.17	0.02	0.42	0.05
Ethyl linoleate				
nonene	0.09	0.01	trace	-
pentyl furan	1.81	0.09	1.60	0.06
Ethyl linolenate				
nonene	0.19	0.02	0.05	0.01

<sup>a</sup>Mean from 2 determinations.<sup>b</sup>Average deviation from the mean.<sup>c</sup>Trace; <0.01  $\mu\text{mol/g}$ .

TABLE 5

Comparison of Decomposition Products of Ethyl Linoleate Heated on Silica and Bulk with the Same Degree of Oxidation at 60°C

Compound	60°				180°			
	Silica <sup>a</sup> ( $\mu\text{mol/g}$ )		Bulk <sup>b</sup> ( $\mu\text{mol/g}$ )		Silica <sup>c</sup> ( $\mu\text{mol/g}$ )		Bulk <sup>d</sup> ( $\mu\text{mol/g}$ )	
	Mean <sup>e</sup>	SD <sup>f</sup>	Mean	SD	Mean	SD	Mean	SD
Et octanoate	0.5	0.1	11.3	1.1	2.0	0.1	10.4	1.0
Et 9-oxononanoate	20.6	1.1	16.3	1.9	22.3	2.3	7.4	1.7

<sup>a</sup>Silica samples were heated 4 hr.<sup>b</sup>Bulk samples were heated 10 hr.<sup>c</sup>Silica samples were heated 1 hr.<sup>d</sup>Bulk samples were heated 40 hr.<sup>e</sup>Mean from 2 determinations.<sup>f</sup>Average deviation from the mean.

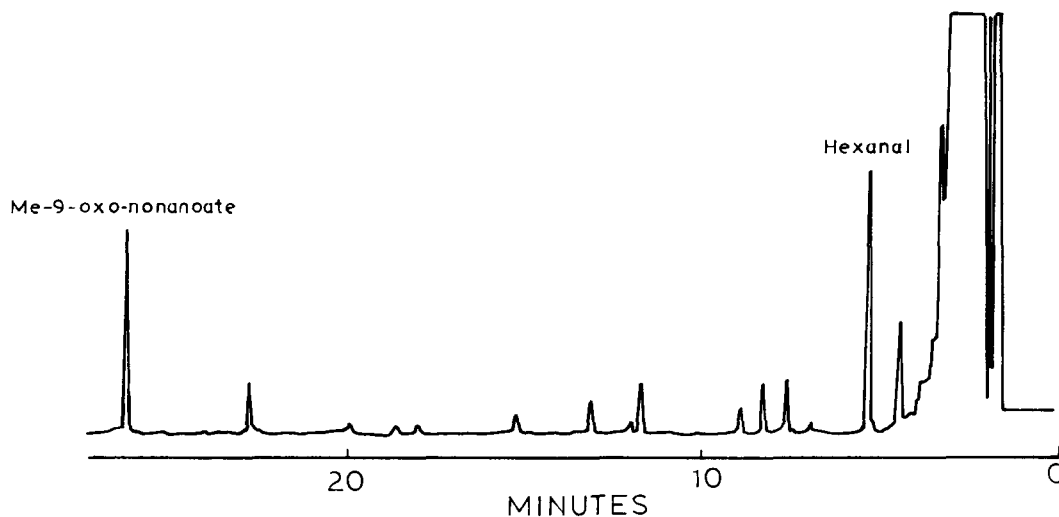


FIG. 2. Gas chromatographic analysis of thermal decomposition products of 9,10-epoxyoctadecenoic acid and 12,13-epoxyoctadecenoic acid.

It is quite possible, therefore, that the volatiles produced in the case of the oxidation on silica were essentially those produced from heterolytic cleavage between the hydroperoxide group and the allylic double bond, in contrast to bulk samples where homolytic cleavage on both sides of the hydroperoxide is the main mechanism (15).

#### ACKNOWLEDGMENT

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