Photochemical Oxidation of Fatty Acid Esters With and Without Chlorophyll.[1,2] Ultraviolet and Infrared Studies of Products

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NUMEROUS studies on the initial stages of autoxidation of various fatty acid esters, with and without added catalytic agents, have been made. In the case of oleate, progress in elucidating the structures of the initial products has been made by Knight *et al.* (27) by using infrared spectrophotometry to determine the formation of *trans* isomers during the autoxidation of methyl oleate. The method is based on the fact that isolated *trans* olefins show an intense absorption in the infrared region at a wavelength of about 10.3 to 10.4 microns whereas *cis* olefins do not. In the study cited however, the autoxidations were all catalyzed by ultraviolet light and the peroxides were not separated from the unoxidized material, that is, the studies were made on the whole mixture. Because of the latter fact it was not certain whether the observed *trans* double bonds were located exclusively in oxidation products (as appeared most probable) or whether the unoxidized oleate had been partially isomerized to a *trans* configuration (27).

Linoleate esters also autoxidize to form hydroperoxides by a free radical chain mechanism involving attack at the methylenic group between the two double bonds (12, 13, 14, 15, 16). Double bond shifts occur, inasmuch as diene conjugation appears initially in proportion to the peroxide formed (4, 9, 12, 13, 20, 30). Estimates of the proportions of conjugated and unconjugated hydroperoxides formed however have been revised; earlier estimates based on incomplete spectral information placed the proportion of conjugated hydroperoxides as low as 70% (9, 30, 31). Such estimates were based on various chemical and spectral evidences (5, 6, 7, 8, 19) and assumptions, some of which have since proved to be equivocal. Further data concerning the ultraviolet and infrared spectra of geometrically isomeric conjugated dienes (22, 32) and of infrared absorption characteristics of linoleate peroxides have led to a more recent conclusion that at least 90% of conjugated hydroperoxides are present among the initial products of linoleate oxidation at low temperature, primarily in a *cis,trans* configuration (10, 35).

Work by other investigators has indicated that the photooxidation of fatty acid esters with ultraviolet light proceeds via a mechanism that is essentially the same as in autoxidation (3, 39). In the photochemical oxidation of fatty acid esters with chlorophyll however, data have been obtained that suggest a somewhat different mechanism (18, 28, 29).

It is the purpose of this report to present further information concerning the structure of the peroxides formed in the oxidation of methyl oleate and methyl linoleate under various conditions, with particular reference to peroxides formed when these esters are oxidized photochemically with chlorophyll. Similarities and differences in the structures obtained under various conditions have been studied primarily by means of ultraviolet and infrared spectrophotometric measurements. Information concerning the mechanism of the photochlorophyll oxidation from kinetic studies will be given elsewhere (25).

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Experimental

Apparatus. The infrared analyses were made with a Beckman IR-2 instrument equipped with special slit drives (40) for use with both rock salt and lithium fluoride prisms (cell, 0.1 mm.). A Beckman DU ultraviolet spectrophotometer (cell 1.0 cm.) was used. A special apparatus (34) was employed for microdistillations to determine the extent of monomer and polymer formation in the oxidations. Hydrogen values were measured by means of an apparatus described by Joshel (23).

In the photooxidations with visible light, with or without chlorophyll, the light source was a 300-watt photoflood bulb in a housing provided with forced ventilation. The light beam passed through a plate glass window, the Pyrex glass bottom of a thermostated water bath, and a 6-inch layer of water (containing 5% alcohol) before it reached the reaction vessel. The temperature of the system was kept in the range 17 to 18°C. with cold tap water circulated through a copper coil in the bath. The reaction flask was fitted with an oxygen inlet (fritted glass dispersion tube), a stirrer, and an outlet provided with an anhydrous calcium chloride absorption tube.

Materials. Methyl oleate (iodine value 84.3, theory 85.7) and methyl linoleate (iodine value 171.7, theory 172.4; less than 0.1% conjugated material) were obtained from the Hormel Foundation. The latter was prepared by a bromination-debromination procedure but was believed to contain no more than 10% of isomers containing *trans* double bonds (35). Infrared absorption at 10.36 microns indicated approximately 10% of *cis,trans* isomer (Figure 2, curve 1).

The solvent n-heptane was freed from unsaturated materials by treating twice with concentrated sulfuric acid, twice with fuming sulfuric acid, and twice with an acid (1 normal) solution of potassium permanganate (saturated). It was then washed with distilled water, dried with anhydrous calcium chloride, and distilled over this dessicant.

The copper soap used as a catalyst in one experiment for the oxidation of methyl linoleate was prepared from corn oil. A clear aqueous solution of the potassium soaps was treated with an excess of an aqueous cupric sulfate solution, and the precipitate was filtered off, washed several times with water, washed with 80% alcohol twice, and dried to a fine powder.

The crude chlorophyll was obtained from fresh spinach leaves and freed from the other plant pig-

TABLE I

Oxidation of Methyl Oleate
Properties of Peroxide Concentrates and Reduction Products

Sample	Temperature of oxidation	Other conditions of oxidation	P.V.	I.V.	H_2 absorbed	—OH
	°C.		*m.e./kg.*		*mole/mole*	*mole/mole*
1. Peroxide concentrate	25 to 30°	Dark and no catalysis	5640	.....	....	....
2. 1 reduced			104	80.4	1.0	1.0
3. Peroxide concentrate	17 to 18°	Photooxidation with visible light	6035	.....	....	....
4. 3 reduced			52	80.9	1.0	1.0
5. Peroxide concentrate	—1 to —2°	Photooxidation with chlorophyll and visible light	5770	.....	....	....
6. 5 reduced			77	81.5	1.0	1.0
7. Peroxide concentrate	35°	Ultraviolet radiation	5470	.....	....	....
8. 7 reduced			54	.....	....	....
9. Theory for methyl oleate hydroperoxide			6090	.....	....	....
10. Theory for reduced hydroperoxide			0	81.3	1.00	1.00

ments in order to retain only chlorophylls a and b. The methods are described in detail elsewhere (25).

Oxygen-free nitrogen (commercial nitrogen purified by passage over heated copper) was used to purge solutions and reagents and to blanket the products obtained in the oxidations during all manipulations involved in their separation and analysis.

Procedures. In general, between 20 and 30 g. of fatty acid ester were oxidized under each of the sets of oxidation conditions employed.

Methyl oleate was oxidized to peroxide values of 500 to 700 milliequivalents per kilogram under the following conditions: a) autoxidation in air at 25 to 30°C. for about four months, b) oxidation of a 30% solution in heptane in visible light at 17 to 18°C. for about four weeks, c) photochlorophyll oxidation of a 30% solution in heptane at —1 to —2°C. for about 10 hours, d) oxidation in ultraviolet light at 35°C. for about five days. In the first three cases oxygen was bubbled slowly through the liquid medium; in d) a thin layer of oleate was exposed to air.

Methyl linoleate was oxidized to peroxide values of about 600 milliequivalents per kilogram under the following conditions: a) autoxidation in air at 0 to 3°C. for about nine weeks; b) oxidation of a 30% solution in heptane in visible light at 17 to 18°C. for three days; c) photochlorophyll oxidation of a 30% solution in heptane at —1 to —2°C. (two samples, 12 and 33 hours, with latter going to peroxide value of approximately 5,000 m.e./kg.); d) oxidation in ultraviolet light at 35°C. for about 24 hours; and e) oxidation in presence of a copper soap at 25 to 30°C. for about 36 hours. In the first of these oxidation took place by exposure to air in an open vessel; in the last four, pure oxygen was bubbled through from a gas-dispenser tube.

The more rapid photochlorophyll oxidations were conducted at lower temperature to minimize the formation of secondary products and the destruction of chlorophyll. The rate of the primary reaction has previously been found to be relatively independent of temperature between 20 and 60°C. (18). The concentration of chlorophyll employed was 2.0 mg. per ml. of substrate.

For oxidations in ultraviolet light, both methyl oleate and linoleate were stirred continuously in open beakers, with an ultraviolet lamp (General Electric AH4) placed about four inches above the surface of the esters, and dried oxygen was passed through with a fritted glass dispersing tube.

The products of the photochlorophyll oxidations were freed from chlorophyll and its oxidation products by passing the reaction mixture into a glass tubing 3 in. in diameter packed to a depth of 3 in. with powdered sugar, and on top of this a depth of 2 in. of charcoal, and then eluted with 800 to 1,000 ml. of heptane containing 5% diethyl ether. This procedure did not change the original peroxide value of the substances appreciably.

The peroxides were quantitatively separated from the unoxidized esters by partition between two immiscible solvent phases (36) made by mixing water, absolute ethyl alcohol, and Skellysolve F in the proportions 7:40:47.

The peroxide concentrates were reduced to the corresponding hydroxyl compounds with four to five times the theoretical amount of stannous chloride (0.5 to 1% in alcohol) at room temperature with 3 hrs. reaction time (35).

The infrared measurements were made on 10% solutions of the materials in either carbon disulfide or tetrachlorethylene, depending on whether the wavelength region under examination was above or below 7.2 microns. For better resolution, a lithium fluoride prism was used for the regions of hydroxyl, carbon-hydrogen, and carbonyl absorptions. Three regions were examined in detail: a) 10.0 to 11.0 microns for geometric isomers of carbon-carbon double bond systems; b) 2.0 to 4.0 microns for hydroxyl, hydroperoxyl, and carbon-hydrogen bonds; c) 5.0 to 7.0 microns for carbonyl groups.

Ultraviolet measurements were made in spectroscopically pure absolute ethyl alcohol at 234 millimicrons to follow diene conjugation.

The hydrogen absorption was estimated as moles of hydrogen per mole of unsaturated substance, using the Joshel hydrogenator (23) with a 50-ml. burette and 0.5 g. of methyl oleate, or 0.25 g. of methyl linoleate, or the products from them in equivalent amounts in 25 ml. purified dioxane (17) in the presence of platinum catalyst (2). With pure linoleate, values were obtained by this method which deviated from theoretical values by less than 5%.

The Wijs iodine value method (½ hour) was used for the methyl oleate products and the Woburn method (41) for the methyl linoleate products. Hydroxyl values were determined essentially by the method of Ogg *et al.* (33).

Results and Discussion

Peroxides from methyl oleate. Table I shows the analytical data obtained on the peroxide concentrates from oxidized methyl oleate and on their reduced products. The hydrogen values, iodine values, and hydroxyl values of the reduction products confirm the formation of a large proportion of monohydroperoxides at the level of oxidation employed. The presence of peroxides other than hydroperoxides re-

cently reported by Swern *et al.* (39) is not evident from, but also not precluded by, these data. The data are consistent with previously reported results based on the use of deuterium tracer techniques (24). Samples 1, 5, and 7 showed somewhat lower than the theoretical peroxide values for pure monohydroperoxides, indicating the presence of some products which, according to the peroxide method employed, were nonperoxidic. Sample 3, oxidized with plain light, showed a nearly theoretical peroxide value.

Infrared analysis revealed two absorption bands of special interest in peroxides from oleate and their reduced products (Figure 1). Both types of compounds absorb at 2.8 to 2.9 microns (hydroxyl stretching vibrations) and at 10.36 microns (bending vibrations of carbon-hydrogen groups attached to a *trans* double bond). The hydroxyl absorption band parallels the content of peroxidic and other hydroxyls and was found to be in agreement with the findings of previous workers (1, 11, 21, 35), including the observation that hydroxyl absorption occurs a a lower wavelength than does hydroperoxyl. These absorption curves are not included because they were all similar to those of the products of methyl linoleate (Figures 2, 3).

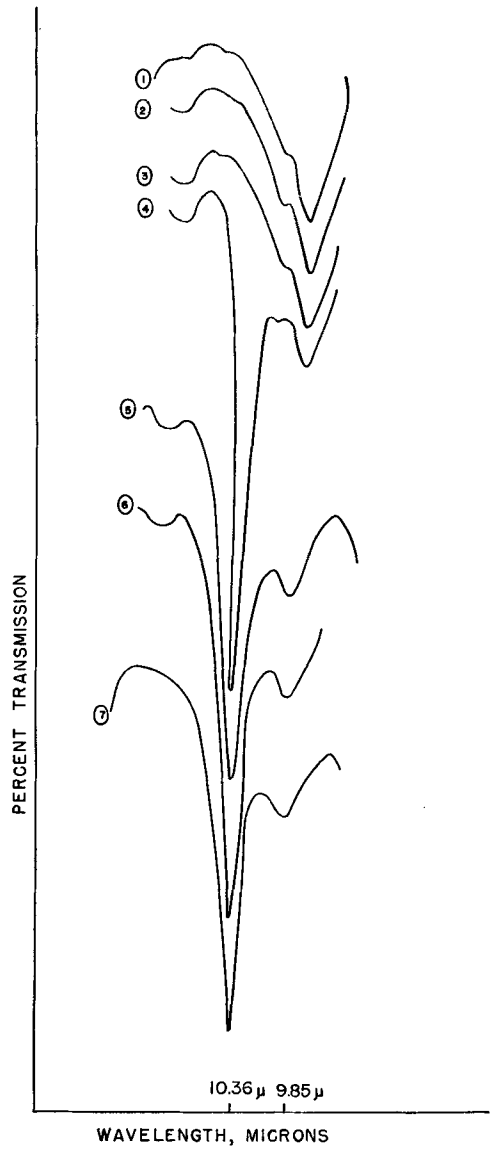

FIG. 1. Infrared absorption spectra. (1) Methyl oleate, (2) Recovered unoxidized material from the sample oxidized with chlorophyll plus visible light at −1 to −2°C., (3) Recovered unoxidized material from the sample oxidized with visible light only at 17 to 18°C., (4) Methyl elaidate, (5) Peroxide concentrate from methyl oleate oxidized at 25 to 30°C., (6) Peroxide concentrate from methyl oleate oxidized with visible light at 17 to 18°C., (7) Peroxide concentrate from methyl oleate oxidized with chlorophyll plus visible light at −1 to −2°C.

The difficulties encountered by Knight *et al.* (27) due to overlap of the the 9.8 micron methyl ester band with the 10.36 micron band of *trans* double bonds was largely overcome in the present study due to the prior separation of the peroxides from unoxidized material. Because of this separation also it was demonstrated that the unoxidized oleate was not isomerzied measurably to a *trans* form (note absence of 10.36 micron band in curve for unoxidized oleate in Figure 1), thus eliminating mechanisms which include such a process (27).

Our results confirm those of Knight *et al.* (27) in that the reduced peroxides showed essentially the same amount of *trans* double bonds as the original peroxides. Also the peroxides were predominantly of *trans* configuration, but the amount of *trans* isomer varied appreciably under different conditions of oxidation. Thus estimations by means of the base-line technique indicated that the peroxides from uncatalyzed autoxidation (sample 1) contained about 75% *trans* forms, those from oxidation in visible light (sample 3) about 90%, those from the photochlorophyll oxidation (sample 5) 97 to 100%, and those from the ultraviolet catalyzed oxidation (sample 7), about 67%. These estimates are based on comparisons with the absorption of methyl elaidate, assuming a constant molecular extinction coefficient for an isolated *trans* double bond.

The peroxide concentrate from the photochlorophyll oxidation was solid at −50°C. whereas the others were viscous liquids at the same temperature. Microdistillation (34) of the reduced peroxide concentrates of autoxidized and photochlorophyll-oxidized oleate show that these products are mostly monomeric in character (97% to 100% monomer).

Peroxides from methyl linoleate. In Table II analytical data are given for the peroxide concentrates obtained from methyl linoleate oxidized under the several sets of conditions, and for their reduced products.

Autoxidation at about 0°C. in the dark yielded a peroxide concentrate having almost the theoretical peroxide value for a monohydroperoxide, also exhibiting a higher spectral absorption at 234 millimicrons than the products obtained under other conditions. These data are consistent with the view that the peroxides formed in the autoxidation of methyl linoleate are almost completely conjugated (35).

Photooxidation with visible light at 17 to 18°C. yielded products with somewhat lower ultraviolet spectral absorption, and catalysis by copper soaps in the dark at 25-30°C. yielded a peroxide concentrate with significantly lower peroxide value and ultraviolet absorption.

The most striking difference however was found in the products of the photochlorophyll oxidation; the peroxide concentrate exhibited an almost theoretical peroxide value and at the same time a decidedly

TABLE II

Oxidation of Methyl Linoleate
Properties of Peroxide Concentrates and Reduction Products

Sample	Temperature of oxidation	Conditions of oxidation	P.V.	I.V. (Wodburn)	H_2 absorbed	—OH	Absorptivity or specific ext. coeff.
	°C.		*m.e./kg.*		*mole/mole*	*mole/mole*	
1. Peroxide concentrates	0 to 3°	Dark and no catalysis	6100	.....	....	....	77.0
2. 1 reduced			60	174.7	2.0	1.0	77.3
3. Peroxide concentrates	17 to 18°	Photooxidation with visible light	5980	.....	....	....	66.9
4. 3 reduced	17 to 18°	Photooxidation with visible light	300	173.2	....	....	70.7
5. Peroxide concentrate	—1 to —2°	Chlorophyll and visible light	5940	.....	....	....	44.9
6. 5 reduced	—1 to —2°	Chlorophyll and visible light	47	169.3	2.0 (I.V. 163)	1.0	41.5
7. Peroxide concentrate	35°	Ultraviolet light	5560	.....	....	....	63.1
8. 7 reduced	35°	Ultraviolet light	107	157.9	....	1.0	62.8
9. Peroxide concentrate	25 to 30°	Dark and 1% copper soap	5470	.....	....	....	64.3
10. 9 reduced	25 to 30°	Dark and 1% copper soap	55	.....	....	....	62.9
11. Calculated for linoleate hydroperoxide			6115	.....	....	....	99.5[a], 86[b]
12. Calculated for reduced hydroperoxide			0	163.5	2.00	1.00	102.5[a], 90[b]

[a] For *trans,trans* conjugated.
[b] For *cis,trans* conjugated.

lower spectral absorption at 234 millimicrons. Further evidence described below, particularly from infrared analysis, indicated that this was in large measure due to the formation of appreciable quantities of unconjugated peroxides.

Several types of methyl linoleate isomers that are related to the structures formed in the autoxidation of methyl linoleate show important absorption bands in the infrared region. Jackson *et al.* (22) found that the pure *cis,trans* conjugated isomer showed a doublet with absorptions at 10.55 and 10.18 microns (curve 4, Figure 1) and pure *trans,trans* conjugated isomer showed a single strong band at 10.12 microns (curve 3), but no band at 10.55 microns. Mixtures of *cis,trans* and *trans,trans* have bands at 10.55 and 10.15 microns (21, 22). For the purposes of this study the several types of isomers involved could be distinguished by examination of the spectra at three wavelengths: 10.15 microns (*trans,trans* conjugated), 10.36 microns (nonconjugated or isolated *trans*), and 10.55 microns (*cis,trans* conjugated). These bands are designated A, B, and C respectively in Figure 2.

Infrared data for various peroxide concentrates and for reduced concentrates are given in Figures 2 and 3, and the pertinent observations are summarized in Tables III and IV. The infrared data in Figure 2 reveal that autoxidation at low temperature caused the formation predominantly of *cis,trans* conjugated hydroperoxides. Photooxidation with visible light, ultraviolet catalyzed oxidation, and copper catalyzed oxidation yielded mainly *trans,trans* conjugated forms. Similarly a sample of methyl linoleate autoxidized at approximately 24°C. yielded predominantly *trans,trans* conjugated isomers.

The photochlorophyll-oxidized samples however exhibited a considerable absorption at 10.36 microns, indicating that some isolated *trans* double bonds were present in this case. *Trans* double bonds were present in the original methyl linoleate, but increased intensity of absorption showed that most of those present were formed during the oxidation. This was also shown to be true on the basis of supplementary studies involving the photoxidation of methyl esters of corn oil fatty acids in the presence of chlorophyll under similar conditions; in these cases *trans* double bonds were originally completely absent. Moreover in the latter study, a compound containing the isolated *trans* double bond in a reduced linoleate peroxide concentrate has subsequently been isolated by

TABLE IV

Oxidation of Methyl Linoleate
Infrared Absorption (cf. Figure 3)

Curve no.	Substance	*cis,trans* conjugated Band A 10.55 microns	*trans,trans* conjugated Band C 12.12 microns	Isolated *trans* Band B 10.36 microns
1.	Sample 5, Table III, reduced	++	+++	....
2.	Sample 7, Table III, reduced	+	+++	....
3.	Sample 8, Table III, reduced	+	++	+
4.	Sample 7, Table II, reduced	+	+++	....
5.	Sample 9, Table II, reduced	+	+++	....

TABLE III

Oxidation of Methyl Linoleate
Infrared Absorption (cf. Figure 2)

Curve no.	Substances	Temperature of oxidation	Condition of oxidation	*cis,trans* conjugated Band A 10.55 microns	*trans,trans* conjugated Band C 12.12 microns	Isolated *trans* Band B 10.36 microns
		°C.				
1	Original methyl linoleate		.....	.....	.....	+
2	Recovered unoxidized linoleate			.....	.....	+
3	Unoxidized conjugated *trans,trans* linoleate			.....	+++	....
4	Unoxidized conjugated *cis,trans* linoleate			++	+++	....
5	Methyl linoleate peroxide	0 to 3°	Dark and no catalysis	++	+++	....
6	Methyl linoleate peroxide[a]	24°	Dark and no catalysis	+	+++	....
7	Methyl linoleate peroxide	17 to 18°	Photooxidation of 30% solution in heptane by visible light	+	+++	....
8	Methyl linoleate peroxide[a]	3 to 5°	Photooxidation of 30% soln. in heptane by chlorophyll and visible light	+	++	+
9	Methyl linoleate peroxide	—1 to —2°	Photooxidation by chlorophyll and visible light (12 hrs.)	+	++	+
10	Methyl linoleate peroxide	—1 to —2°	Photooxidation by chlorophyll and visible light (33 hrs.)	+	++	+

[a] Samples obtained in supplementary experiments not described in text.

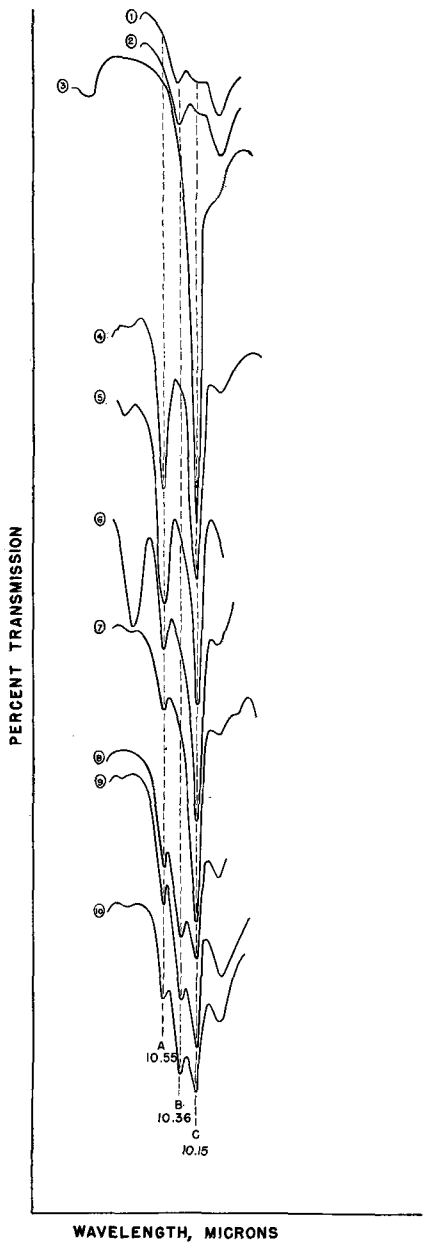

FIG. 2. Infrared absorption spectra. (1) Methyl linoleate, (2) Recovered unoxidized linoleate, (3) Conjugated *trans, trans* methyl octadecadienoate, (4) Conjugated *cis, trans* methyl octadecadienoate, (5) Peroxide concentrate from methyl linoleate oxidized at 0 to 3°C., (6) Peroxide concentrate from linoleate oxidized at 24°C., (7) Peroxide concentrate from linoleate oxidized in visible light at 17 to 18°C., (8) Peroxide concentrate from linoleate oxidized with chlorophyll and light at 3 to 5°C., (9) Peroxide concentrate from linoleate oxidized with chlorophyll in light at −1 to −2°C. (12 hours), (10) Peroxide concentrate from linoleate oxidized with chlorophyll in light at −1 to −2°C. (33 hours).

displacement chromatography (26). Other studies with esters of corn oil acids further demonstrated that oleate and linoleate in mixtures are oxidized relatively nonselectively in the photochlorophyll oxidation, confirming observations previously made in this laboratory (18, 29).

Spectra of these oxidation products in the rock salt region from 2.0 to 7.0 microns are almost the same as those published previously (35), indicating strong and characteristic hydroperoxyl or hydroxyl absorption, carbon-hydrogen absorption, and carbonyl absorption. Lithium fluoride prism spectra for the carbonyl region (Figure 4) showed all of the products to be virtually identical except the product from photochlorophyll oxidation, which did not show a band at 5.80 microns.

The reduced peroxides showed a stronger OH band at 2.92 microns as a thin film (curve 2, Figure 5) than as a 10% solution (curve 1, Figure 5), due to weaker absorption of dissociated OH in solution.

The LiF spectra (Figure 4) shows a strong band at 3.30 microns in unoxidized linoleate (curve 1), which is very weak in all of the peroxide concentrates (curves 2 to 6). This band increases in intensity as the number of isolated *cis* double bonds increases in the series stearate, oleate, linoleate, linolenate, and arachidonate. The isolated *trans* double bond of elaidate contributes little to this band (37). We have confirmed this on elaidate and have observed that

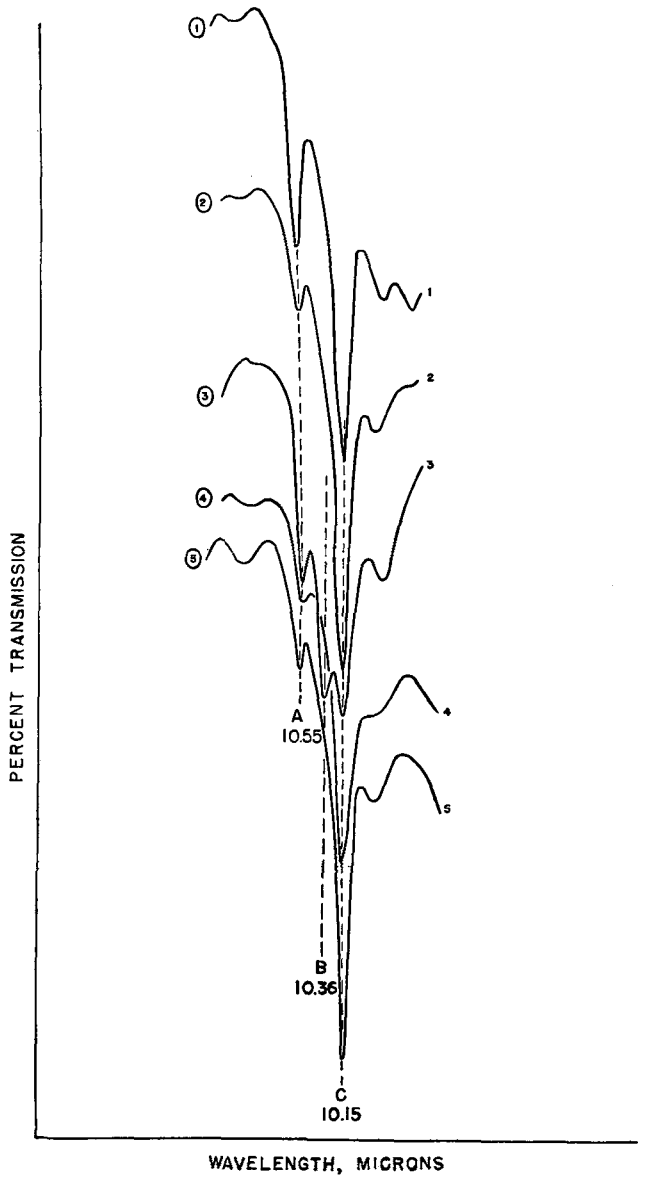

FIG. 3. Infrared absorption spectra. Reduced peroxide concentrates from methyl linoleate oxidized (1) at 0 to 3°C., (2) at 17 to 18°C. with visible light, (3) at 3 to 5°C. with chlorophyll and visible light, (4) at 35°C. with ultraviolet light, (5) at 25 to 30°C. with 1% copper soap.

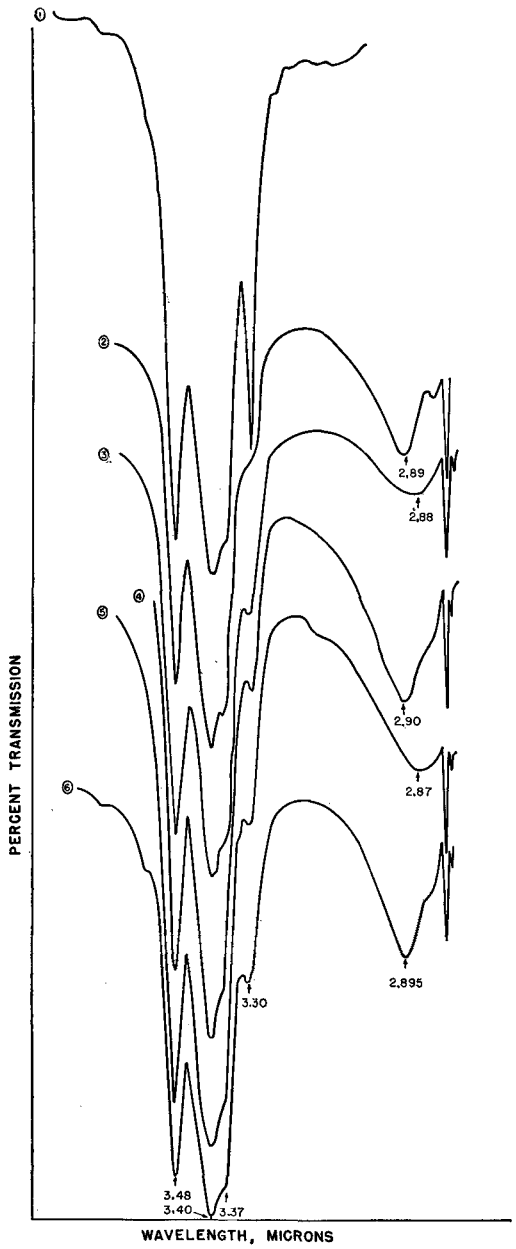

FIG. 4. Infrared absorption spectra (lithium fluoride prism and C_2Cl_4 solvent). (1) Methyl linoleate, (2) Peroxide concentrate from linoleate oxidized at 0 to 3°C. without solvent, (3) Reduced (2), (4) Peroxide concentrate from linoleate oxidized at 17 to 18°C. with visible light, (5) Reduced (4), (6) Peroxide concentrate from linoleate oxidized at −1 to −2°C. with chlorophyll and visible light.

linoelaidate (*trans,trans* nonconjugated) is similar to stearate and elaidate in its band intensity at 3.30 microns. Conjugated linoleates (both *cis, trans* and *trans,trans*) have a band about equal to *(cis)* oleate at 3.30 microns. Thus it appears that this band is associated with nonconjugated isolated *cis* double bonds due either to the carbon-hydrogen group of the R—CH=CH—R structure (37), or the carbon-hydrogen of CH_2 groups adjacent to double bonds (1, 35, 38). The band in the linoleate peroxide concentrates is only one-third to one-half as strong as in unoxidized oleate or unoxidized conjugated linoleate. This fact suggests that the CH_2 assignment of the 3.30 micron band is more reasonable (35).

In general, the findings of the present investigation indicate that photooxidation with visible light, ultraviolet catalyzed oxidation, and copper soap catalyzed oxidation, like autoxidation, yield primarily conjugated hydroperoxides. Moreover, at higher temperatures or in the presence of light, *trans,trans* conjugated forms predominate. Only in the case of the photochlorophyll oxidation does any appreciable amount of unconjugated hydroperoxide appear to be formed, and part of this appears to contain *trans* double bonds. However, even in the latter case, the principal products are *trans,trans* conjugated forms, even at 0°C.

Summary

1. It has been confirmed that the principal products formed in the oxidation of methyl oleate by oxygen under a variety of conditions are predominantly *trans* hydroperoxides. However no inversion of the double bond occurs in unoxidized oleate. Hence the conversion of *cis* to *trans* double bonds and peroxide formation occur together in the same molecules.

2. The autoxidation of methyl linoleate at low temperature yields predominantly *cis,trans* conjugated hydroperoxides. Autoxidation at 25°C., oxidation catalyzed by visible light, or ultraviolet light and copper soap catalyzed oxidation at temperatures appreciably above 0°C., lead to the formation primarily of *trans,trans* conjugated hydroperoxides. The inversion of the second double bond in this case appears to be independent of the peroxide-forming reactions.

3. The photochlorophyll oxidation of methyl linoleate leads to the formation of some unconjugated

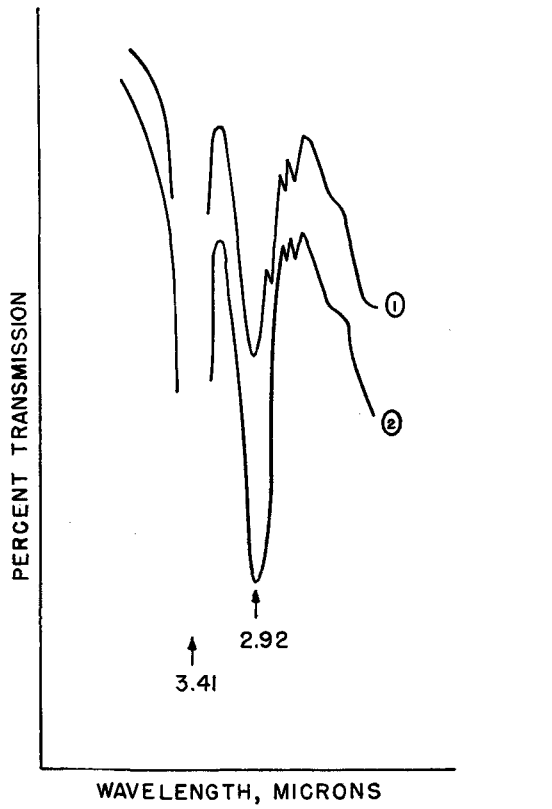

FIG. 5. Infrared absorption spectra. Reduced peroxide concentrates from methyl linoleate oxidized with ultraviolet light at 35°C.: hydroxyl region for (1) 10% solution, (2) thin film.

hydroperoxides, some of which contain *trans* double bonds.

4. Under all of the conditions employed in the present investigation, the oxidation of methyl oleate and linoleate led primarily to the formation of monomeric peroxides which retained most of the unsaturation of the parent compound.

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The Determination of Monoglycerides and Glycerin in Mixtures

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THE most satisfactory methods of determining monoglycerides and glycerin are based upon their quantitative oxidation with periodic acid according to the following equations:

$$CH_2OH - CHOH - CH_2OR + H_5IO_6 \longrightarrow CH_2O + CHO - CH_2OR + HIO_3 + 3H_2O$$

Monoglyceride

$$CH_2OH - CHOH - CH_2OH + 2H_5IO_6 \longrightarrow 2CH_2O + HCO_2H + 2HIO_3 + 5H_2O$$

Glycerin

In each reaction, quantitative oxidation can be obtained if an excess of periodic acid is used. The amount of monoglyceride or glycerin is determined by measuring the amount of periodic acid consumed. Glycerin can also be determined by titration of the formic acid that is produced (6).

Several variations in procedure have been proposed (1, 2, 3, 4, 5). Each of the methods has one or more of the following disadvantages: a) high-melting samples must be warmed, resulting in side reactions; b) the sample must be extracted with water or a salt solution to remove free glycerin, if any is present; c) the sample and oxidizing reagent are in separate liquid phases which must be kept mixed by constant stirring; and d) when the excess of periodic acid is determined (addition of potassium iodide and titration with sodium thiosulfate), the precision of the titration is limited because the sample titration must be equal to at least 80% of the blank titration.

In the methods in current use the sample of monoglyceride, dry or dissolved in chloroform or ethyl acetate, is added to a measured amount of a solution of periodic acid dissolved in 80% or 95% acetic acid. The excess periodate remaining after the reaction is complete is reduced to iodate, and all of the iodate (including that formed during the oxidation reaction) is reduced to iodine by the addition of potassium iodide to the acid solution. The amount of iodine formed is measured by titration with sodium thiosulfate.

$$H_5IO_6 + 2HI \longrightarrow HIO_3 + I_2 + 3H_2O$$

$$HIO_3 + 5HI \longrightarrow 3I_2 + 3H_2O$$

$$4I_2 + 8Na_2S_2O_3 \longrightarrow 8NaI + 4Na_2S_4O_6$$

The difference between the blank and sample titrations therefore represents the amount of periodate which had been reduced to iodate by the monoglyceride. By this method three-fourths of the oxidizing power is spent in the reduction of iodate to iodine. If stoichiometric amounts of monoglyceride and periodate were used, the sample titration would be 75% of the blank. Since a 20% excess of periodic acid is advised, the sample titration is at least 80% of the blank. Therefore, in order to obtain a reasonable difference in the two titrations, it is necessary to know in advance (or by preliminary analysis) the approximate percentage of monoglyceride.

The characteristic feature of this proposed method is the determination of periodate using conditions