

g. (30%) of 2,3,4-trimethoxybenzyl *t*-butyl ether (X) as a colorless oil, b.p. 91° (0.20 mm.),  $n_D^{25}$  1.5043.

*Anal.* Calcd. for  $C_{14}H_{22}O_4$ : C, 66.11; H, 8.72. Found: C, 66.01; H, 8.38.

**Kinetic Data.**—A reaction of 10.0 g. (0.0335 mole) of VI in 30.0 ml. of cyclohexanone at  $114 \pm 1^\circ$  showed the following nitrogen evolution: time in minutes (volume of nitrogen in ml.); 5(42), 10(110), 15(175), 20(230), 25(290), 30(345), 35(390), 40(430), 50(500), 60(545), 80(615), 120

(705), 240(775). A similar experiment with only 19 ml. of cyclohexanone gave essentially identical results. A plot of these data in terms of % reaction vs. log time gives a curve almost identical with that constructed from the equation for series first-order reactions in which it is assumed that  $k_1$  and  $k_2$  are approximately equal.<sup>22</sup>

(22) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1953, pp. 153-157.  
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[CONTRIBUTION FROM THE HORMEL INSTITUTE, UNIVERSITY OF MINNESOTA]

## Displacement Analysis of Lipids. IX. Products of the Oxidation of Methyl Linoleate<sup>1</sup>

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RECEIVED NOVEMBER 23, 1953

The peroxides formed by six different means of oxidation of methyl linoleate have been isolated, reduced to the corresponding hydroxy compounds and subjected to displacement analysis. The products obtained *via* autoxidation at  $-10^\circ$  in the dark, with copper catalyst, with visible light irradiation, or with ultraviolet light irradiation are qualitatively similar. The two major dienoic reduction products exhibit *cis-trans* and *trans-trans* conjugation. With chlorophyll photooxidation, four major reduction products were found. One was found to exhibit no conjugation, and to have hydroxyl groups and isolated *trans* double bonds. It was found to have two double bonds and one hydroxyl group per molecule. This compound could be 11-hydroxylinoleate derived from the non-conjugated peroxide. The products of oxidation produced by lipoxidase oxidation to a level of 25% were found to consist of at least six substances distinguishable by displacement chromatography diagram. It is concluded that displacement analysis is a sharp analytical tool for the study of the products of fat oxidation.

Recent studies of the oxidation of esters of linoleic acid have shown that the primary product of oxidation probably exists as more than one isomer.<sup>2</sup> In the most generally accepted mechanism of oxidation of linoleate the free radical formed by removal of a labile hydrogen atom at carbon 11 exists as a resonance hybrid to which addition of oxygen takes place at the ends of the resonating system. This addition of oxygen leads to formation of hydroperoxides containing conjugated double bond systems. Current theories of oxidation of linoleate have been summarized recently.<sup>3</sup> Although no direct evidence has been reported for the existence of non-conjugated peroxide formed by oxidative attack at carbon 11, its existence has not been ruled out. Recently the presence of such non-conjugated peroxides among the products of oxidation of linoleate was suggested by infrared spectra of peroxide concentrates obtained under certain conditions of oxidation.<sup>4</sup>

In the field of lipid analysis, displacement chromatography has been shown to be very useful for the small-scale separation of closely related substances. The applications of displacement analysis to lipids have been summarized recently.<sup>5</sup> Briefly stated, long chain aliphatic compounds have been shown to be separable if they differ from each other by one carbon atom in length,<sup>6</sup> type of functional group,<sup>7a</sup> kind and degree of unsaturation<sup>7b</sup> and by branching

or polar groups along the chain. It seemed likely then that displacement analysis might be used to separate the closely related products of oxidation of methyl linoleate, and that displacement diagrams of these products might yield additional information regarding the complexity of these products formed under differing conditions of oxidation. Moreover, isolation and characterization of the suspected non-conjugated peroxide might be possible.

### Experimental

Methyl linoleate, prepared by bromination-debromination, was obtained from the Hormel Foundation. Methyl esters of corn oil acids were prepared by methanolysis and used for confirmatory experiments. Oxidation of these ester preparations was performed under six differing conditions: (A) autoxidation at  $-10^\circ$  in darkness, (B) autoxidation with copper oleate catalyst, (C) autoxidation under visible light irradiation, (D) autoxidation under ultraviolet light irradiation, (E) oxidation in the presence of chlorophyll and under visible irradiation and (F) "enzymatic" oxidation with lipoxidase. The details of these oxidations have been described elsewhere.<sup>4,8</sup> Analytical data pertinent to these preparations and later treatment are summarized in Table I. The linoleate which had been oxidized by various means was subjected to countercurrent extraction to isolate the peroxide components.<sup>9</sup> These peroxide concentrates were then reduced by stannous chloride to obtain the monohydroxylinoleate preparations which were subjected to displacement chromatography.

The chromatographic apparatus and its use have been described in detail elsewhere.<sup>10</sup> The system used in the separations reported here consisted of a coupled filter column of 76.2 ml. total volume packed with Darco-G60 and Hyflo Supercel, 1:2. The solvent was 95% ethanol and the displacer solution was 1.0% ethyl stearate. Samples of reduced oxidation products varied from 250 to 440 mg. The effluent from the chromatographic column in all cases, except C, was passed through a quartz flowing cell having a cell thickness of 0.2 mm. mounted in the cell holder of a Beckman DU spectrophotometer. Ultraviolet light absorption was measured at 2520 Å., which represents principally end

(1) Supported in part by contract with the Office of Naval Research (N8onr 66218), Department of the Navy. Hormel Institute publication no. 97.

(2) O. S. Privett, W. O. Lundberg, N. A. Khan, W. E. Tolberg and D. H. Wheeler, *J. Am. Oil Chem. Soc.*, **30**, 61 (1953).

(3) R. T. Holman, "Progress in the Chemistry of Fats and Other Lipids," Vol. II, Pergamon Press, London, 1954, p. 51.

(4) N. A. Khan, W. E. Tolberg, D. H. Wheeler and W. O. Lundberg, *J. Am. Oil Chem. Soc.*, in press.

(5) R. T. Holman, "Progress in the Chemistry of Fats and Other Lipids," Vol. I, Pergamon Press, London, 1952, p. 104.

(6) R. T. Holman and L. Hagdahl, *J. Biol. Chem.*, **182**, 421 (1950).

(7) (a) R. T. Holman, *This Journal*, **73**, 3337 (1951); (b) R. T. Holman and W. T. Williams, *ibid.*, **73**, 5285 (1951).

(8) N. A. Khan and W. O. Lundberg, *J. Am. Oil Chem. Soc.*, in press.

(9) O. S. Privett, W. O. Lundberg and C. Nickell, *ibid.*, **30**, 17 (1953).

(10) R. T. Holman and L. Hagdahl, *Anal. Chem.*, **23**, 794 (1951).

TABLE I  
ANALYSES OF METHYL LINOLEATE HYDROPEROXIDE AND METHYL HYDROXYLINOLEATE PREPARATIONS

Preparation	Conditions of oxidation	P.V., meq./kg.	I.V.	H <sub>2</sub> /mole	OH/mole	E <sub>1cm</sub> <sup>1%</sup> 2340
Methyl linoleate hydroperoxide	Dark, -10°	6078				727
Methyl hydroxylinoleate (A)		62	171.7		1.0	738
Methyl linoleate hydroperoxide	Visible light, 18°	5980				669
Methyl hydroxylinoleate (C)		300	173.2			707
Methyl linoleate hydroperoxide	Chlorophyll visible light, -1°	5940				449
Methyl hydroxylinoleate (E)		47	169.3	2.0	1.0	415
Methyl linoleate hydroperoxide	U.V., 35°	5560				631
Methyl hydroxylinoleate (D)		107	157.9		1.0	628
Methyl linoleate hydroperoxide	Cu, 25-30°	5470				643
Methyl hydroxylinoleate (B)		55				629
Methyl linoleate hydroperoxide	Lipoxidase, 0°	>5000				
Methyl hydroxylinoleate (F)		115				794
Methyl linoleate hydroperoxide	Theory	6115				?
Methyl hydroxylinoleate	Theory	0	163.5	2.0	1.0	?

absorption of the conjugated diene chromophore of sufficient intensity that the maximum expected optical density be near unity. The fractions from experiment C were diluted with ethanol and the relative optical density was measured at 2350 Å., near the characteristic maximum for conjugated diene.

Fractions comprising major zones in the chromatograms were combined and evaporated to dryness in vacuum in those cases where further inspection of the isolated substances was planned. In some cases the fractions were subjected to infrared and ultraviolet analysis as well as quantitative hydrogenation. The infrared analyses were

TABLE II  
CHARACTERIZATION OF PRODUCTS OF OXIDATION OF METHYL LINOLEATE BY INFRARED SPECTRA

		Fraction			
		1	2	3	4
<i>Autoxidation at -10° (A)</i>					
Hydroxyl	2.82 μ	Strong	Strong	Strong	
Isolated <i>trans</i> double bond	10.28 μ	....	....	....	
Conjugated <i>trans-trans</i> -diene	10.12 μ	....	Present	Strong	
Conjugated <i>cis-trans</i> -diene	10.18, 10.55 μ	Strong	Strong	Weak	
	11.3 μ	?	?	?	
<i>Ultraviolet irradiation (D)</i>					
Hydroxyl	2.82 μ	Strong	Strong	Strong	Weak
Isolated <i>trans</i> double bond	10.12 μ	?	....	....	....
Conjugated <i>trans-trans</i> -diene	10.18, 10.55 μ	....	Present	Strong	Strong
Conjugated <i>cis-trans</i> -diene	11.3 μ	....	Strong	Present	....
		....	Present	Present	....
<i>Visible light irradiation (C)</i>					
Hydroxyl		Strong	Strong		
Isolated <i>trans</i>		....	....		
<i>trans-trans</i>		Strong	Very strong		
<i>cis-trans</i>		Weak	Weak		
	12.85 μ	....	Strong		
	11.3 μ	Weak	Weak		
<i>Corn oil methyl esters with chlorophyll and visible irradiation</i>					
Hydroxyl		Weak	Strong	Strong	
Isolated <i>trans</i>		Strong	Weak	....	
<i>trans-trans</i>		....	Strong	Strong	
<i>cis-trans</i>		....	Weak	Weak	
	12.85 μ	....	....	....	
	11.3 μ	....	Present	Present	
	11.6 μ	Strong	Present	Present	
<i>Corn oil methyl esters, visible light irradiation</i>					
Hydroxyl		Strong	Strong	Strong	
Isolated <i>trans</i>		?	....	....	
<i>cis-trans</i>		?	Weak	....	
<i>trans-trans</i>		?	Strong	Strong	
	11.3 μ	....	Weak	Strong	

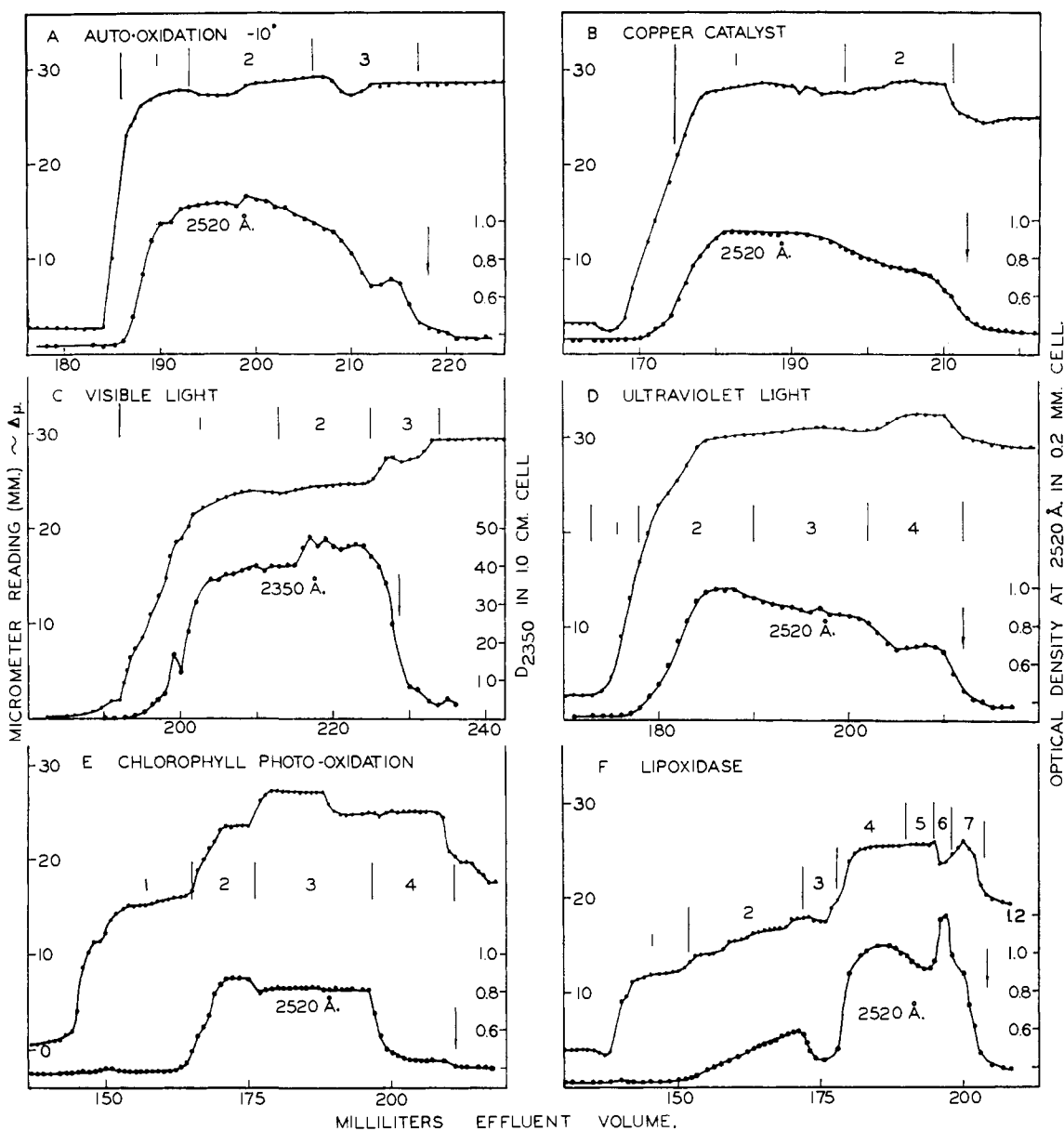


Fig. 1.—Displacement diagrams for six preparations of methyl hydroxylinoleate obtained by stannous chloride reduction of the peroxides isolated from oxidized methyl linoleate. Sample sizes: A, 250 mg.; B, 250 mg.; C, 250 mg.; D, 250 mg.; E, 400 mg.; F, 400 mg. Upper curves, interferometric measurements; lower curves, ultraviolet absorption at indicated wave lengths. Arrows indicate first fraction which deposited crystals at  $-16^{\circ}$ .

performed by D. H. Wheeler and W. E. Tolberg of General Mills Laboratories on thin film sandwiches using a Beckman infrared spectrophotometer with a sodium chloride prism. For interpretation of infrared spectra of fats and fatty acids the reader is referred to a discussion by Wheeler.<sup>11</sup>

**Results**

The displacement diagrams for typical runs of the products of oxidation of methyl linoleate by six different means are shown together in Fig. 1. It is readily seen that the "monohydroxylinoleate" in no case is a single substance. The curves for refractive indices and density at 2520 Å. are generally similar for the preparations obtained *via* autoxidation

at  $-10^{\circ}$ , in the presence of copper catalyst, or under ultraviolet irradiation. With these three preparations and the one obtained through radiation with visible light, it is evident that the major components are conjugated unsaturated substances. The two main components in each of these preparations (1 + 2 vs. 3 of A; 1 vs. 2 of B; 1 vs. 2 of C; 2 + 3 vs. 4 of D) are distinguishable because of the difference in their ultraviolet light absorption. In these four preparations the occurrence of other components is negligible.

**Autoxidation at  $-10^{\circ}$  (A).**—Infrared spectra of the three fractions indicate that fraction 1 is principally *cis-trans*-hydroxyoctadecadienoate, and fraction 3 is principally the *trans-trans* isomer.

(11) D. H. Wheeler, "Progress in the Chemistry of Fats and Other Lipids," Vol. II, Pergamon Press, London, 1954, p. 268.

Fraction 2 consists of a mixture of these. None of the three fractions showed the presence of isolated *trans* double bonds, but all contained hydroxyl groups. The infrared data are summarized in Table II.

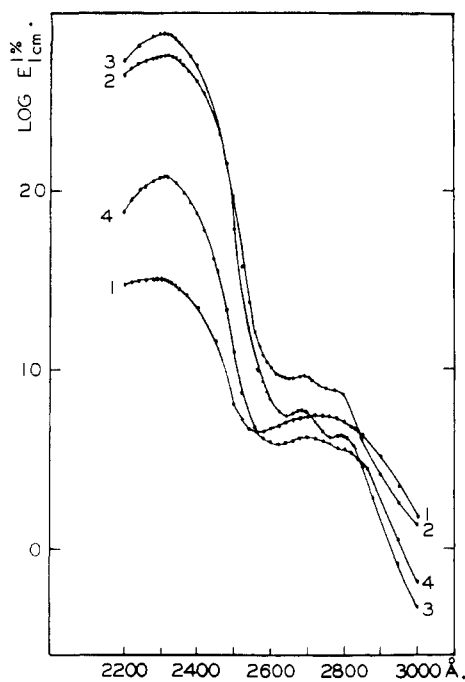


Fig. 2.—Quantitative ultraviolet absorption spectra of the four fractions separated from reduced methyl linoleate peroxide prepared by photochlorophyll oxidation.

**Autoxidation at 35° in the Presence of Copper (B).**—The displacement diagram of the copper-autoxidized preparation indicates that most of the substance was conjugated diene. A very small amount of non-absorbing material preceded the first main fraction. The displacement diagram was very similar to those of other autoxidized materials (A, C, D). Fraction A was found to have  $E_{1\text{cm}}^{1\%}$  at 2330 Å. = 643 and portion B had  $E_{1\text{cm}}^{1\%}$  at 2310 Å. = 818.

**Irradiation with Visible Light (C).**—The first two fractions exhibited strong absorption for *trans-trans* conjugation in the experiment with methyl linoleate; *cis-trans* conjugation was much weaker than in the corresponding early fractions of the autoxidation sample. Unfortunately fraction 3 was insufficient for analysis. Infrared spectra of fractions from a similar experiment with corn oil methyl esters were very similar to these, and a third fraction showed strong absorption at 11.3  $\mu$ , possibly due to epoxy groups.

**Ultraviolet Irradiation (D).**—The four fractions indicated as usual that some overlapping of zones takes place. The major zones 2 and 3 consisted principally of *cis-trans* and *trans-trans* conjugated substances, respectively. The infrared spectra suggest that another chromophoric material may be present which was not observed in the low temperature autoxidation. This suggests that departure from the most gentle autoxidation treatment induces some side reactions.

**Chlorophyll Photooxidation (E).**—The products of this catalytic oxidation have occupied most of our attention because in this case is found strong evidence of additional products of oxidation. The displacement diagram is more easily interpreted because of the sharpness of the separation. The chromatogram consisted of four major zones as marked on a typical experiment, Fig. 1E. The first zone showed no ultraviolet absorption at 2520 Å., whereas the second and third did. The ultraviolet absorption of the fourth zone was found to be low. For further characterization the corresponding zones from three chromatograms were pooled. Quantitative ultraviolet absorption spectra on the four substances are shown in Fig. 2. The infrared spectra of the four fractions are shown in Fig. 3, and the properties are summarized in Table III.

TABLE III  
CHARACTERIZATION OF PRODUCTS OF OXIDATION OF METHYL LINOLEATE WITH CHLOROPHYLL PHOTOOXIDATION (SEE FIG. 1E)

	Fraction			
	1	2	3	4
Hydrogenation				
Sample mg.	18.8	20.7	19.5	20.8
Theory for monohydroxylinoleate, ml.	2.71	2.99	2.82	3.0
Observed	2.56	2.83	2.62	3.08
M.p. hydrogenated esters, °C.	27-32	<30	<27	32-35
Ultraviolet absorption maxima				
$E_{1\text{cm}}^{1\%}$ at 2280 Å.	31.6			
2320 Å.		580		
2310 Å.			758	120
Infrared spectra				
Hydroxyl, 2.82 $\mu$	Strong	Strong	Strong	....
Isolated <i>trans</i> , 10.28 $\mu$	Strong	Present	....	Weak
Conjugated <i>trans-trans</i> , 10.12 $\mu$	....	Strong	Strong	Weak
Conjugated <i>cis-trans</i> , 10.18, 10.55 $\mu$	....	Weak	Weak	....
11.3 $\mu$	....	....	Strong	....

Fraction 1 was found to exhibit strong hydroxyl absorption at 2.82  $\mu$ , strong absorption at 10.28  $\mu$  due to isolated *trans* double bonds, no absorption due to conjugation either in the ultraviolet or infrared, and to have approximately two double bonds per molecule. Inasmuch as the original peroxide values of the peroxide concentrate and the reduced peroxide concentrate were so close to theoretical for methyl linoleate hydroperoxide and methyl hydroxylinoleate, respectively, such a large component in the hydroxylinoleate as the substance in fraction 1 must have arisen from linoleate hydroperoxide.

Parallel experiments performed on corn oil methyl esters gave strictly comparable preparations. Three chromatograms were run and fractions from them were pooled. Hydroxyl value on fraction A of this preparation was found to be 165, whereas the expected value for monohydroxy methyl linoleate is 180. Thus the preparation was demonstrated to possess one hydroxyl group per molecule. Elementary analysis of the preparation indicated: C, 72.4; H, 10.8; by difference, O, 16.8. Theory for methyl hydroxylinoleate is: C, 72.5; H, 10.97; O, 15.5. Methyl dihydroxylinoleate: C, 70.0; H, 10.5; O, 19.5. Methyl linoleate: C, 77.5; H, 11.55; O, 10.95. The analysis corresponds most closely to methyl monohydroxy-

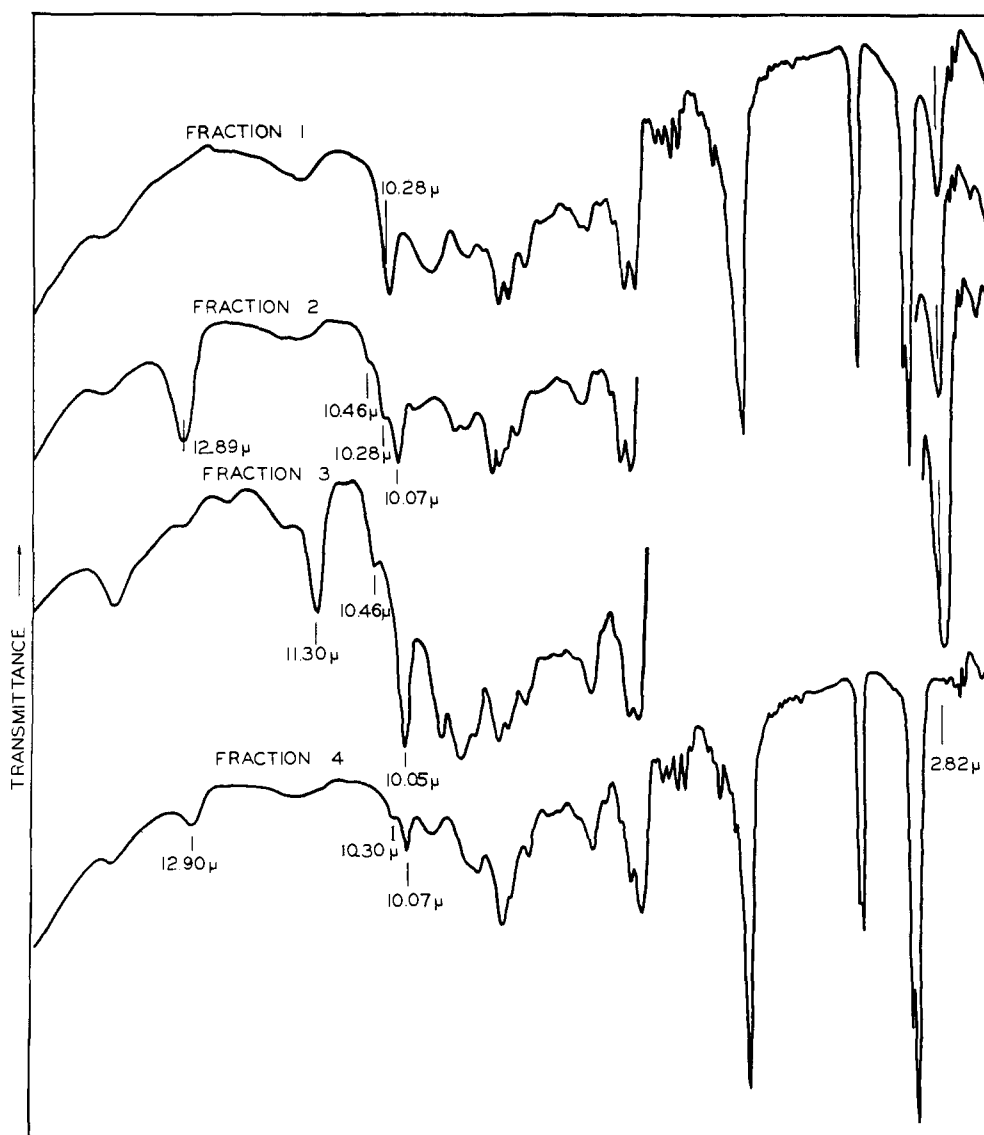


Fig. 3.—Infrared spectra of the four fractions separated from reduced methyl linoleate peroxide prepared by photochlorophyll oxidation.

linoleate and is in harmony with the other characterizations. The substance was subjected to alkaline isomerization, but the degree of conjugation induced under the standardized procedure was low. However, evidence of diene conjugation was deduced from the spectra of isomerized samples. The observed properties of the substance suggest that it is methyl 11-hydroxylinoleate and that it was derived from methyl 11-hydroperoxidolinoleate. Localization of the hydroxyl group will involve X-ray analyses and comparisons with known hydroxy stearate. These facilities are not at present available to the authors.

Fractions 2 and 3 both exhibit strong *trans-trans* conjugation and only weak evidence of any *cis-trans* conjugation. The low ultraviolet extinction coefficient may be explained by the admixture of substance 1. The general absence of *cis-trans* conjugation agrees with the proposition that the initial product of oxidation of linoleate is *cis-trans* but

that this product probably is converted to the *trans-trans* isomer by elevated temperature and other conditions.<sup>2</sup> The interferometric data and ultraviolet absorption data taken during the chromatographic separation suggest strongly that substances 2 and 3 are distinct substances, for the transition between zones involved a sharp increase in refractive index increment but a decrease in absorption at 2520 Å. Inasmuch as both are *trans-trans* conjugated substances, this may indicate separation of the 9,11- and 10,12-isomers.

Fraction 4 represents a substance having more than two double bonds and negligible absorption due to conjugated diene or isolated *trans* double bonds. Its identity is not known.

**Lipoxidase Oxidation (F).**—Information is less complete regarding the substances separated from this preparation. The displacement diagram, however, does show that the preparation contained at least 6 distinct substances. However, the oxida-

tion of the linoleate had been carried to about 0.25 mole O<sub>2</sub> per mole of linoleate, which is relatively high, and some of the zones in the chromatogram may represent secondary oxidation products. It appears from the displacement diagram that some of the products exhibit low ultraviolet absorption and are thus not the primary products of oxidation, which appear to be conjugated.<sup>12</sup> It should also be pointed out that the enzyme preparation used was soybean extract, and it could conceivably contain enzymes capable of promoting secondary reactions. However, it may be concluded from this experiment that displacement chromatography is a very useful tool for the separation and isolation of the product of oxidation of linoleate, being able to separate a large number of closely related substances.

#### Discussion

To the authors' knowledge, the isolation of what appears to be 11-hydroxylinoleate from reduced peroxides, represents the first chemical evidence for the existence of the non-conjugated peroxide of linoleic acid among its oxidation products. Bergström<sup>13</sup> concluded from his studies on autooxidation of methyl linoleate that the non-conjugated peroxide did not occur. He chromatographed on alumina the hydroxy stearates obtained by hydrogenation of the peroxides. The 9- and 13-hydroxystearates were isolated and identified, but no 11-

(12) S. Bergström and R. T. Holman, *Nature*, **161**, 55 (1948).

(13) S. Bergström, *Arkiv Kemi Mineral. Geol.*, **A21**, 14 (1945).

hydroxy stearate could be demonstrated. This was not taken as proof that the non-conjugated isomer did not exist, because isomerization could have taken place during the hydrogenation. However, in the present investigation, the appearance of the non-conjugated substance in some preparations and not in others, when all were treated in identical ways, speaks strongly for the absence of the non-conjugated peroxide among the products of autooxidation. The non-conjugated product has been conclusively demonstrated only in chlorophyll-photooxidation which is believed to proceed by a mechanism different from that in autooxidation, and which is not a chain reaction.<sup>8</sup> It is possible also that zone 1 of the lipoxidase preparation could be this substance.

The preliminary experiments described here demonstrate the applicability of displacement analysis to problems involving the products of oxidation of fats or fatty acids. A segregation of *cis-trans* and *trans-trans* conjugated dienes has been shown in all cases, although the separation of these is not as sharp as between these and other products of oxidation. In general, the separation of substances has been verified by the infrared spectra of the fractions wherever this means of identification has been applied.

The authors wish to acknowledge the technical assistance of Herbert Hayes.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF DELAWARE]

## Fatty Acid Esters of 3-Butene-1,2-diol. II.<sup>1</sup> The Copolymerization of Erythryl Dilinoleate with Styrene<sup>2</sup>

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RECEIVED OCTOBER 24, 1953

Erythryl dilinoleate was polymerized with styrene by heating under nitrogen at 80° in the presence of benzoyl or *p*-chlorobenzoyl peroxide to give low yields of linear, soluble polymers containing from 2.8 to 28 mole % of erythryl dilinoleate units. Limiting viscosity numbers varied from 7.6 to 12.6. The ester did not homopolymerize to high polymers. Saponification of two of the polymers and analysis of the main chain indicated that approximately nine-tenths of the linkages by which the ester was attached to styrene involved the erythryl rather than the linoleic unsaturation. Polymers containing more than 12 mole % of the ester were cross-linked by heat treatment.

A number of recent papers<sup>4-8</sup> have been concerned with the polymerization of allyl and substituted allyl esters of fatty acids. However, limited attention<sup>1</sup> has been given to esters of polyhydric alcohols containing the allyl structure. The present investigation of the linoleic acid diester of erythryl (3-butene-1,2-diol) was undertaken to determine whether the compound is homopolymerizable or

copolymerizable with styrene and to study the linkages found in such polymerization.

The nature of unsaturation and polyfunctionality in erythryl dilinoleate, CH<sub>2</sub>=CHCH(OCOC<sub>17</sub>H<sub>31</sub>)CH<sub>2</sub>(OCOC<sub>17</sub>H<sub>31</sub>), offered rather unusual possibilities for polymerization. Polymerization through the allylic unsaturation, with or without styrene, would yield a linear chain with linoleic acid residues as branches. Such a structure should be convertible to a three-dimensional cross-linked polymer, by reaction of the linoleic unsaturation. Furthermore, it was of interest to know the extent to which the linoleic unsaturation could enter into linear copolymerization.

The linoleic acid was obtained from safflower seed oil<sup>9</sup> as the ethyl ester. Conversion to eryth-

(1) Paper I, E. Dyer, T. G. Custer and W. C. Meisenhelder, *THIS JOURNAL*, **71**, 2728 (1949).

(2) Based upon the Ph.D. dissertation of George A. Weisgerber, University of Delaware, 1951.

(3) Armstrong Cork Company Research Fellow, 1948-1951.

(4) P. D. Bartlett and R. Altschul, *THIS JOURNAL*, **67**, 812, 816 (1945).

(5) (a) D. Swern, G. N. Billen and H. B. Knight, *ibid.*, **69**, 2439 (1947); (b) D. Swern and E. F. Jordan, *ibid.*, **70**, 2334 (1948); (c) D. Swern, *ibid.*, **71**, 2377 (1949).

(6) S. A. Harrison and D. H. Wheeler, *ibid.*, **73**, 839 (1951).

(7) E. Dyer and W. C. Meisenhelder, *ibid.*, **73**, 1434 (1951).

(8) N. G. Gaylord and F. R. Birch, *ibid.*, **74**, 337 (1952).

(9) Of the total fatty acids in the oil, 76.7% was normal linoleic acid, as shown by ultraviolet spectrophotometric analysis. Thanks are due to the Chemurgy Project, University of Nebraska, for generously supplying the safflower seed oil.