The Isolation and Characterization of the Polymers Formed During the Autoxidation of Ethyl Linolenate[1,2]

L. A. WITTING,[3] S. S. CHANG,[4] and F. A. KUMMEROW, Department of Food Technology, University of Illinois, Urbana, Illinois

PREVIOUS ATTEMPTS to characterize the autoxidative polymers of linolenates have met with little success since, after oxidation, scission products, partially oxidized monomers, polymers, and unreacted ester form a complex mixture which is not easily resolved (4). It has also been pointed out that many of the accepted quantitative determinations of functional groups may give erroneous results when applied to oxidized fats (17, 27). In the present work the autoxidation products of pure ethyl linolenate were fractionated by solvent extraction. The polymer fractions were then analyzed by procedures designed to overcome many of the errors frequently noted in determinations of the constants of autoxidized fat.

Experimental

Ethyl linolenate was prepared from raw linseed oil by the bromination-debromination method as described by Rollett (23) and Kass (15). To follow the oxidation, a closed system was used, which, except for the lack of a sigmamotor (Figure 1), was
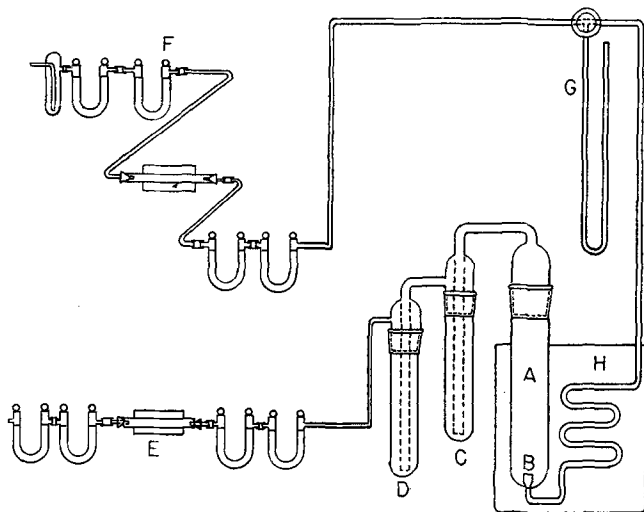

FIG. 1. Diagram of apparatus used for autoxidation of the ester in a closed system: A. reaction vessel; B. fritted glass stick; C. cold trap; D. cold trap; E. absorption train; F. absorption train; G. manometer; and H. constant temperature bath.

similar to that described by Johnson, Chang, and Kummerow (14). The sample, 500 g. of ethyl linolenate, was placed in the reaction tube A and suspended in a constant temperature bath H, which was maintained at 30° ± 1°C. Oxygen was dispersed through the ethyl linolenate by means of a fritted

glass filter B, sealed into the bottom of the tube. The reaction tube was connected to two cold traps, C and D, which were cooled with a mixture of dry ice and acetone. These traps in turn were connected to an absorption train E, in which gaseous decomposition products were collected. The oxygen entering the system was freed of CO_2, H_2O, CO, and H_2 by means of purification train F. Oxygen was blown through the system continuously from a standard K type cylinder.

At various intervals the autoxidation was interrupted, the amount of volatile decomposition products in C and D and gaseous products in E was determined, and a sample was removed for analysis (Table I). The autoxidation was terminated when the refractive index of the autoxidized material had reached a maximum. The polymerized ethyl linolenate was stored at —20°C. under nitrogen prior to analysis.

A solvent-extraction procedure, essentially that of Chang and Kummerow (4), was employed to separate the polymers from the monomers and scission products formed during the autoxidation. The separation was made by use of solvent mixtures of diethyl ether and Skellysolve F. (Table II). After storage under nitrogen at —20°C. for three months, fraction 12 was further subdivided into five new fractions by extraction with solvent pairs made up of redistilled acetone and Skellysolve F. The analyses herein reported were made upon the arbitrarily selected fractions 9 and 12-II.

Infrared absorption spectra were determined with the use of a Perkin-Elmer double beam spectrophotometer. Samples were analyzed as smears between two plates of sodium chloride. Ultraviolet absorption spectra were determined with a Beckman model D. U. spectrophotometer. Absolute ethanol and 0.5% aqueous potassium hydroxide in absolute ethanol were used as solvents.

The molecular weights of the samples were determined in two ways. First it was done cryoscopically in benzene, using a Precision-Shell cryoscopic molecular weight apparatus. Then when it had been ascertained that the two fractions, 9 and 12-II, were essentially equivalent to the dimer and trimer, a second value was obtained, based on ethoxyl group analysis. The procedure of Elek and of Samsel and McHard was used in the manner suggested by Siggia (24). The peroxide value was determined by the method of Fugger et al. (10). The hydroxyl group was determined by the method of Chang and Kummerow (4). This analysis was specifically designed for use on oxidized fats. Epoxy groups were determined by the method of Swern et al. (28).
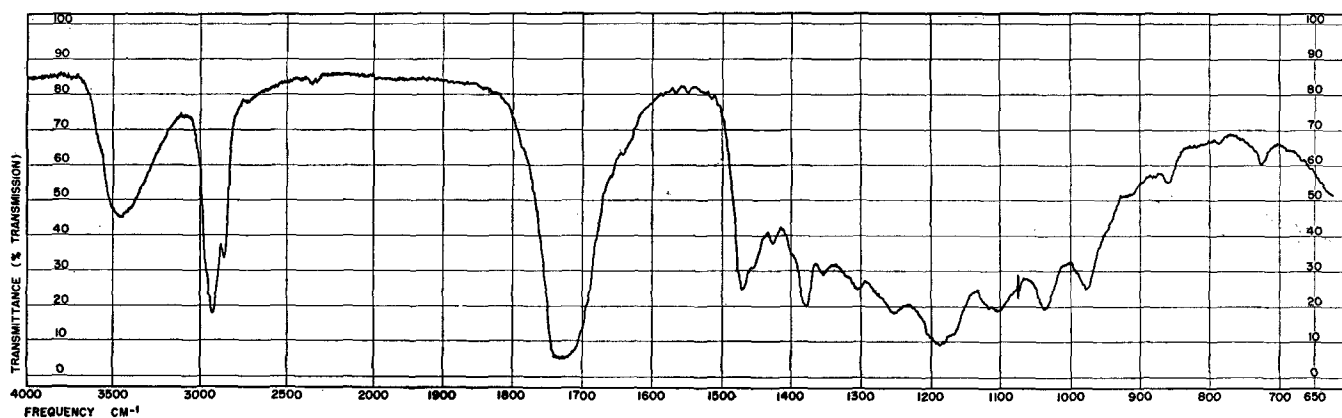
The method of Bryant and Smith (3) was used to determine the ketone carbonyl groups. Hydrogenation at 15 p.s.i.g. at room temperature over a platinum catalyst for one hour was used as a preliminary treatment for removal of the peroxide group prior to analysis for carbonyl groups. The values so obtained

The absorption spectra of the dimer fraction in the infrared region.
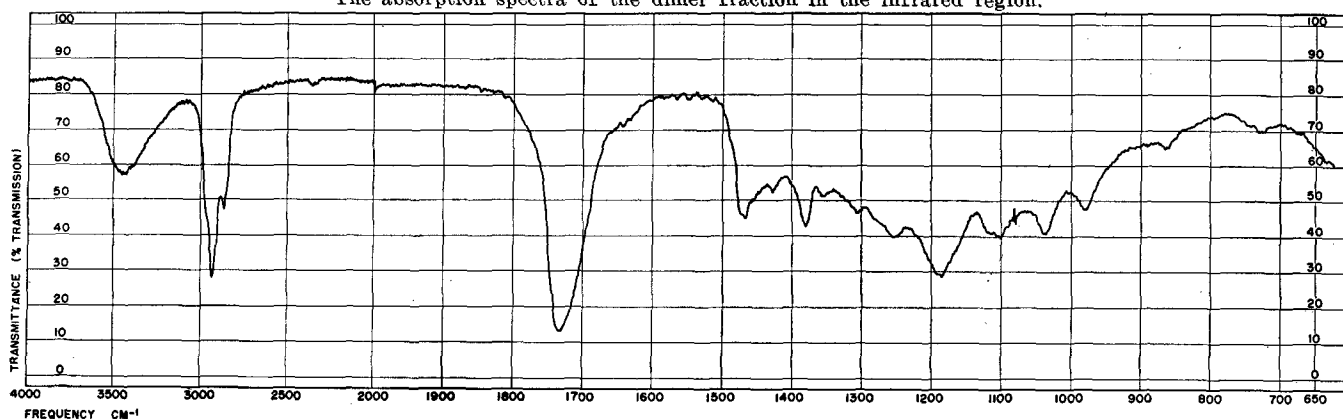
The absorption spectra of the trimer fraction in the infrared region.

FIG. 2

were corroborated by their agreement with the observations of Treibs (30) on samples which were not hydrogenated. The hydrogen number, corrected for the peroxide value, was used as a measure of unsaturation.

Results

In the course of the oxidation the refractive index of the ethyl linolenate increased from 1.4658 for the pure ester to 1.4841 for the autoxidized product. This increase in the refractive index indicated that there was an increase in the number of functional groups and an increase in molecular weight of the autoxidized ester. Carbon dioxide production reached a maximum at about 60 hrs., coincident with the first qualitative detection of carbonyl compounds in the cold traps. As the reaction progressed, the peroxide number increased while the iodine number decreased (Table I).

Refractionation of fraction 12, after three months' storage under nitrogen at —20°C., indicated that

even under these conditions some spontaneous decomposition had occurred. Two main fractions were obtained, which were distinguished by their difference in solubility in 20% acetone in Skellysolve F. Three small (0.5, 0.8, and 0.7 g.) fractions soluble in Skellysolve F containing 0, 5, and 10% acetone were also obtained. Refractive indices indicated that these materials were degradation products. These fractions were not subjected to further analysis.

In the infrared region the absorption spectra of fractions 9 and 12-II were almost identical (Figure 2). On examination of the spectra, absorption bands characteristic of the methyl group, the methylene group, carbon to carbon double bond, and the carbonyl group could be detected. The ketonic carbonyl group, shown to be present by chemical analysis, could not be resolved from the ester carbonyl group in the infrared region. Dugan et al. (5) have pointed out that absorption resulting from the ketone carbonyl group appears as an indefinite shoulder on the

TABLE I

Decomposition Products Formed and Changes in Constants During
Autoxidation of Ethyl Linolenate

Time (hrs.)	CO_2 (g.)	H_2O[a] (g.)	CO (g.)	H_2 (g.)	Cold traps (g.)	Peroxide No. (millimoles/ kilo.)	Iodine number	Refractive index (30°C.)
0	—	—	—	—	—	0	248.2	1.4658
20	0.0088	.0109	.0043	.0020	0.07	228	234	1.4658
40	0.1951	.0047	.0009	.0003	0.01	390	229	1.4679
60	0.8464	.0038	.0221	.0008	0.09	1054	208	1.4711
102	0.1392	.0062	.1120	.0126	0.48	1608	147	1.4822
130	0.0136	.0065	.0336	.0159	0.36	1488	111	1.4841
150	0.0042	.0048	.0387	.0089	0.31	1753	118	1.4840

[a] Water in cold traps not included.
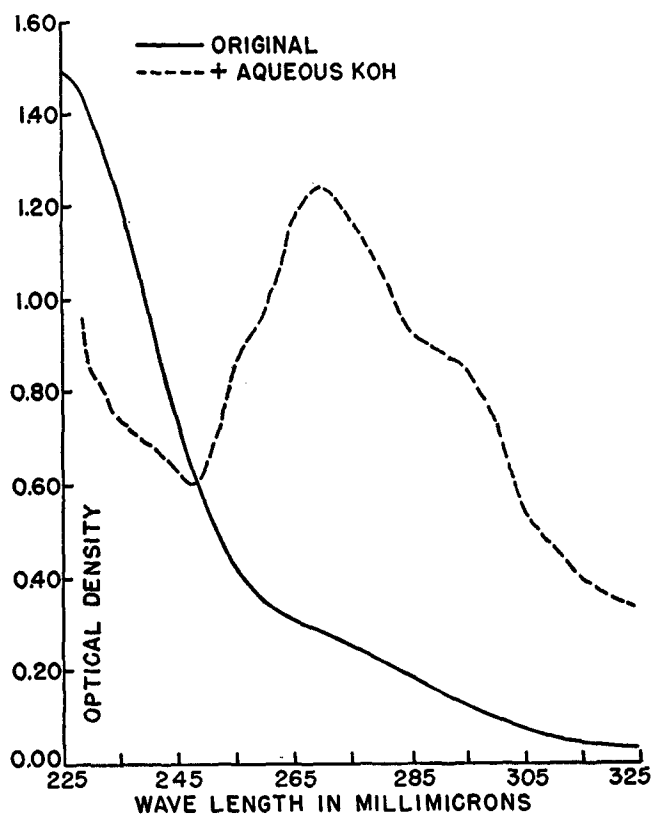
The absorption spectra of the dimer fraction in the ultraviolet region.
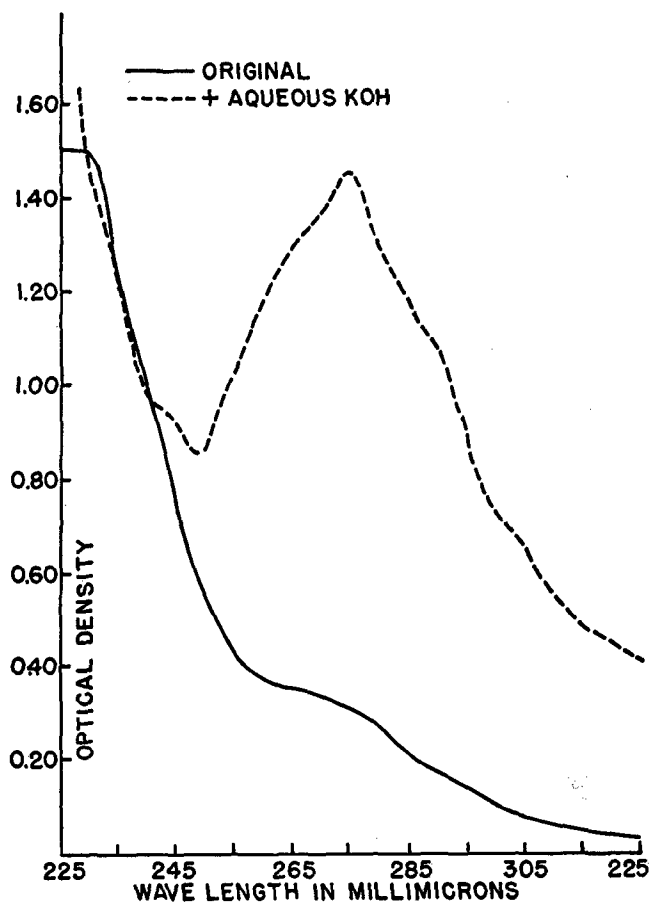
The absorption spectra of the trimer fraction in the ultraviolet region.

FIG. 3

TABLE II
Polymer Fractions Obtained on Solvent Extraction

Fraction	Ethyl ether %	Skelly-solve F. %	Weight (g.)	Ref. index (28°C.)	Peroxide No.
1	0	100	89.3	1.4701	731
2	2	98	12.6	1.4855	2008
3	4	96	17.0	1.4824	2150
4	6	94	5.3	1.4812	1576
5	8	92	3.9	1.4810	1622
6	10	90	5.4	1.4811	1728
7	12	88	12.2	1.4829	1660
8	20	80	6.6	1.4842	1618
9	30	70	21.0	1.4854	1563
10	40	60	34.4	1.4880	1381
11	50	50	30.6	1.4899	1401
12	60	40	31.0	1.4917	1403
13	70	30	27.5	1.4950	1381
14	80	20	8.1	1.4960	1318
15	90	10	8.0	1.4985	....
16	100	0	3.9	Solid	....

band, which was caused by ester carbonyl in autoxidized linoleate. Dugan et al. considered the band at 3467 cm.[-1] as due to hydroxyl groups and that at 3430 cm.[-1] as due to peroxides. It is of interest to note that these absorption spectra are similar to those obtained from the polar polymers of ethyl linoleate and methyl linoleate hydroperoxide.

The absorption spectra in the ultraviolet region indicated that neither the dimer (Figure 3) nor the trimer contained two, three, or four double bonds in conjugation. Treatment with mild alkali developed a maximum at 2750 Å, which was probably caused by a conjugated system of three double bonds formed through the enolization of an alpha diketone in conjugation with a double bond. A scheme whereby this type of alpha diketone could arise during the autoxidation of unsaturated fatty esters has been postulated by Morrell and Phillips (19). That an alpha diketone was present was further indicated by the development of a strong reddish-brown color when alcoholic solutions of the dimer or the trimer fraction were treated with potassium hydroxide. This coloration, according to O'Daniel and Parsons (20), resulted from the quinoid compounds which are formed by aldol condensation of the alpha diketones in a manner analogous to the formation of paraxyloquinone from diacetyl.

On a basis of molecular weight and ultimate analysis (Table III), fraction 9 was found to corre-

TABLE III
Characterization of the Polymers

	Dimer	Trimer
Ultimate analysis		
C%..	63.23	62.17
H%..	9.16	9.09
O%..	27.61	28.74
Molecular weight		
Cryoscopic.......................................	758	1169
Calculated on ethoxyl group.........	778	1222
Empirical formula.............................	$C_{40}H_{69}O_{12.9}$	$C_{60}H_{107}O_{20.8}$
Double bonds per mole.......................	1.8	3.0

spond to a dimer formed from 2 moles of ethyl linolenate plus 9 atoms of oxygen while fraction 12 corresponded to a trimer formed from 3 moles of ethyl linolenate plus 15 atoms of oxygen.

Since the solvent-extraction procedure did not have the required degree of selectivity to produce ideal fractionation, the components which made up the polymer fractions cannot be considered to have been homogeneous in composition. However the data (Table IV) would appear to indicate that the dimers and trimers differed only in the character of the functional groups attached to a given molecule. Since the hydroxyl and carbonyl groups were probably formed

Functional group	Dimer[a]	Trimer[a]
Peroxide	2.4	3.2
Hydroxyl	1.4	3.4
Carbonyl	3.1	4.0
Ester	4.0	6.0
Epoxy	0.0	0.0
Subtotal	10.9	16.6
Attributed to polymeric linkage	2.0	4.0
Undetected	0.0	0.2
Total	12.9	20.8

[a] Atoms of oxygen per mole of polymer.

by secondary reactions of the peroxide group, this series occurred because secondary reactions of this type had not proceeded to completion. The characterization therefore represented the average state of the polymers within the fraction.

The hydrogenation prior to determination of the carbonyl group cannot be considered a possible source of error. A slight production of carbonyl groups on hydrogenation of lipid peroxides may occur. However the conjoint determination of carbonyl and hydroperoxides with hydroxylamine indicated that this did not occur at a measurable level. Slover and Dugan (25) state that existing carbonyl groups are not measurably reduced by this hydrogenation procedure.

The dimer and trimer fractions, on a basis of ultimate analysis and molecular weight calculations, contained 12.9 and 20.8 atoms of oxygen, respectively. However only 10.9 and 16.6 atoms of oxygen, respectively, were identified through functional group analysis. It is possible that the undetected oxygen may be present in the polymeric linkage.

Discussion

In autoxidative polymerization Holman et al. (12) believed that the carbonyl group could undergo aldol condensation with active methylene groups to form carbon-to-carbon linked polymers. Since oxygen serves to conjugate the nonconjugated systems (8), Farmer strongly advocated the view that polymers are formed by a Diels-Alder type of reaction between conjugated double bonds and a single double bond. This mechanism has been shown to operate during thermal polymerization.

The differences in the solubility properties of autoxidative and thermal polymers seem to indicate that they are not of the same type. Kass (16) pointed out that thermal polymers become progressively insoluble in oxygen-bearing solvents whereas autoxidative polymers become progressively insoluble in nonpolar solvents. Powers, Overholt, and Elm (22) suggested that the polymers are formed by addition at the double bond to give a vinyl type of polymer. This suggestion is also subject to the limitations set by Kass. A second type of polymer that has been proposed is the oxygen-linked polymer. This linkage has been suggested in three forms. Swern (26) favored an ether linkage. Hollis (11) pictures the polymerization reaction as occurring through the combination of epoxide groups to form a dioxane ring while Treibs (29) considered that the ring could be formed by an addition of peroxide groups, followed by a rearrangement of the original complex. The work of Farmer (6, 7, 9) and of Bolland and Gee (2) has indicated that oxidative polymers are most probably peroxide-linked. Farmer has postulated a

scheme in which a conjugated double bond reacts with oxygen by 1:4 addition and this cyclic peroxide reacts to form a polymer by adding to a second cyclic peroxide. Bolland and Gee preferred to consider a free radical mechanism, where a peroxide radical regardless of the nature of its formation adds to a double bond. This free radical mechanism has been supported by Bawn (1) and Powers (21).

From the present data, if all of the undetected oxygen is assumed to be present in the polymeric linkage, two possible structures exist: the dioxane ring or the peroxide bond. Either of these would require 2 atoms of oxygen in formation of a dimer and 4 in formation of a trimer. Similar data were noted by Chang and Kummerow (4) in their studies of the autoxidative polymers of ethyl linoleate.

In an attempt to learn more of the nature of the polymeric linkage the polymeric material was depolymerized with 3.4 N ethanolic HCl, as recommended by Chang and Kummerow (4). The polymer fraction, which had an average molecular weight greater than 758, was depolymerized to a product with an average molecular weight of 296. Insufficient material was available for characterization of the depolymerized product to be completed.

Summary

Pure ethyl linolenate was prepared and autoxidized; and dimeric and trimeric fractions were separated by solvent fractionation. These fractions were subjected to quantitative, functional group-analysis. On a basis of oxygen known to be present but not detected in the functional groups the possibility that polymerization had taken place through an oxygen linkage was considered. The mild nature of the reagent causing depolymerization suggested that this linkage was of the peroxide type rather than the dioxane ring.

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