amount of unsaponifiables found is corrected for the 0.13% originally present in the stearic acid. The data are presented in Table III. Good recoveries were obtained in all cases, and the average deviation from theory is ± 4%. This is equivalent to a standard deviation at the 1% level of unsaponifiable of only 0.05%.

The reproducibility for replicate analyses of three different tallows, a coconut oil, and a soap is presented in Table IV, and the results are compared to the macro method values.

TABLE IV

Unsaponifiable Content of Some Fats

Sample	No. of runs	% Unsap.		
		Semimicro	A.O.C.S. Ca 6a-40	Std. dev.
Tallow A	6	0.79	0.57	0.12
Tallow B	10	0.83	0.55	0.05
Tallow C	2	0.58	0.45	0.04
Coconut oil	4	0.40	0.39	0.05
Soap	3	0.45	0.44	0.04

The semimicro method gave the same or slightly higher unsaponifiable values than the macro procedure, A.O.C.S. Ca 6a-40. Since the semimicro (ion exchange) method is a direct determination of nonionic material whereas the macro (extraction) method is empirical, the semimicro results should be more accurate and more nearly true values. The reproducibility of the semimicro method is good and is comparable to that obtained by the macro method. The standard deviation from the mean for the five samples was 0.06%.

Twenty-three additional determinations on fat and oil samples were run under routine analytical conditions. The standard deviation from the mean for the range 0–2% unsaponifiable matter was 0.06%.

When soaps are examined for unsaponifiables plus unsaponified materials, the sample is dissolved in warm water and extracted with ethyl ether without any saponification. The ion exchange procedure is the same as that in the method.

The unsaponifiable determination is applicable to all normal fats and oils (both crude and refined), to partial glycerides and esters, and to fatty acids and soaps containing no more than 22 carbon atoms. Severely oxidized fats or polymerized fatty acids cannot be analyzed because these fatty acids are not retained by the ion exchange resin. Some modification of the eluting system could possibly give better retentions.

This method has been used in various nutritional studies and should find application in clinical investigations. Where it is desirable to use this technique on a preparative scale or where larger samples are to be used, proportionate increases in reagents and equipment were found to give satisfactory results.

REFERENCES

1. Buerki, C. R., and Holt, K. E., J. Am. Oil Chemists' Soc., *31*, 335 (1954).
2. Cason, J., Sumrell, G., and Mitchell, R. S., J. Org. Chem., *15*, 850 (1950).
3. Fitelson, J., J. Assoc. Offic. Agr. Chemist, *31*, 358 (1948).
4. Mattson, F. H., and Beck, L. W., J. Biol. Chem., *214*, 115 (1955).
5. Sylvester, N. D., Ainsworth, A. N., and Hughes, E. B., Analyst, *70*, 295 (1945).
6. "Official and Tentative Methods of the American Oil Chemists' Society," 2nd ed., 1946, American Oil Chemists' Society, Chicago. Method Ca 6a-40.
7. *Ibid.*, Method G 3-53.

[Received April 28, 1960]

Thermal Dimerization of Fatty Ester Hydroperoxides[1]

E. N. FRANKEL, C. D. EVANS, and J. C. COWAN, Northern Regional Research Laboratory,[2] Peoria, Illinois

FORMATION of conjugated fatty hydroperoxides as initial products in fat autoxidation is well established (1,6,8,22,23,28). However knowledge on the subsequent decomposition of hydroperoxides is relatively incomplete. Some of the confusion in the literature arises from the great variety of conditions of decomposition used by different workers.

Thermal decomposition of fat hydroperoxides produces a variety of scission products (3,10,13,19) and high-molecular-weight material, principally dimeric in nature (7,8,27,31,35,36). When decomposition is carried out at relatively low temperatures and in the presence of oxygen, dimeric products appear to be oxygen-linked. By molecular distillation Swern *et al.* (31) isolated polymeric fractions from methyl oleate autoxidized at 65°C. in the presence of cobalt oleate. To account for the excessive oxygen they believed that the polymers were oxygen-linked and assumed an ether linkage since the polymers were resistant to saponification. Chang and Kummerow (7) and Witting *et al.* (36) used solvent extraction to isolate polymers from ethyl linoleate and linolenate, respectively, which were autoxidized at 30°C. They were able to split the polymers into monomeric units with concentrated halogen acids, thus indicating an oxygen-linkage.

When decomposition of fat hydroperoxides is carried out at temperatures above 100°C. and in an inert atmosphere, the dimeric products appear to be linked by a carbon-to-carbon bond. Williamson (35) decomposed autoxidized methyl linoleate (33°C., U.V. light) by heating it at 100°C. in nitrogen. Dimers isolated by solvent fractionation and molecular distillation contained monomeric units linked by carbon-to-carbon bonds since they were not cleaved with hydrogen iodide. In these studies the isolation procedures were complicated by the presence of incompletely decomposed fat hydroperoxides and other products of autoxidation similar in solubility characteristics to the polymerized material. It is difficult to isolate polymerized material by solvent fractionation of autoxidized mixtures without contamination

---

[1] Presented at 33rd fall meeting, American Oil Chemists' Society, Los Angeles, Calif., Sept. 28–30, 1959.

[2] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

by peroxides. On the other hand, molecular distillation of incompletely decomposed autoxidized fats would be expected to result in further thermal decomposition of fat hydroperoxides.

In the present study the dimeric products (hereafter called dimers) were isolated from autoxidized fats and methyl esters and from purified fatty hydroperoxides after complete decomposition of the hydroperoxides at 210°C. in an inert atmosphere. These conditions are similar to, but not identical with, the commercial processing conditions encountered during deodorization of vegetable oils. The purpose of our work was to characterize the dimers formed under these conditions. Previously these dimers were shown to lower the flavor and oxidative stability of soybean oil (11) and may therefore contribute to the flavor reversion problem.

Experimental

Preparation of Methyl Esters. The methyl esters of safflower oil and soybean oil were prepared by a standard transesterification procedure with sodium methoxide in methanol, followed by vacuum distillation. Methyl linoleate was prepared by esterification of a linoleic acid concentrate derived from safflower fatty acids by Podbielniak extraction with furfural and hexane (2). The fraction boiling at 147–150°/0.5 mm. was used (I.V. 170.0, theoretical 172.2). Gas-liquid chromatography showed the presence of 1.2% oleate. Methyl oleate (99.5+% pure), obtained from Applied Science Laboratories Inc., State College, Pa., had no detectable impurities by gas-liquid chromatography.

Alkali-conjugated methyl linoleate was prepared from safflower fatty acids crystallized from hexane at −40°C. to remove saturated acids. Isomerization was carried out with 15% KOH in ethylene glycol at 190–202°C. for 45 min. The conjugated acids were methylated in methanol and sulfuric acid, and the methyl esters were distilled at 144–160°/40 μ. The conjugated methyl linoleate had an absorptivity of 76.9 at 232 mμ (the corresponding absorptivity of *cis-trans* conjugated methyl linoleate is 93.7) and contained approximately 85% *cis,trans* diene by infrared analysis.

Autoxidation. The soybean oil was autoxidized at 60°C. in the dark with oxygen. The methyl esters were autoxidized at 5°C. by exposure to a high-pressure, mercury-vapor Hanovia lamp (100 watts) and a continuous stream of oxygen. Hydroperoxide concentrates were prepared from autoxidized methyl esters of safflower oil (peroxide value 1,800 me/kg.) by the countercurrent extraction procedure of Zilch *et al.* (37). The purified hydroperoxide fractions had peroxide values ranging from 3,630 to 5,300.

Decomposition. The autoxidized esters were decomposed by heating at 210°C. for 15 to 30 min. with agitation from a fine stream of nitrogen. This treatment was sufficient to destroy all the peroxides as determined iodometrically with potassium iodide. When the peroxide value of the esters exceeded 1,000, the initial stages of decomposition at 210°C. were very vigorous, and it was necessary to treat the esters in small lots of 50 to 100 g. The volatiles were collected through an air condenser and consisted of water and liquid products. Decomposition of autoxidized soybean oil was carried out under the conditions of laboratory steam deodorization: 210°C., 2 hr., pressure below 1 mm. Hg.

Preparation of Dimers. The dimers were distilled from thermally decomposed autoxidized esters at 5–15 μ in a molecular still. The deodorized-autoxidized soybean oil was converted to methyl esters prior to distillation. Typical fractionations are given in Table I.

TABLE I

Molecular Distillation of Thermally Decomposed Autoxidized Fatty Esters

Fractions	Temperature, °C.	Percentage of total	Molecular weight
Cyclic still.[a] Safflower methyl esters (P.V. 1525). Pressure: 15–50 μ. Two passes at each temperature.			
1	100	41.2	301
2	120	5.0	336
3	150	5.7	421
4	175	5.9	517
5	200	7.3	568
6	225	5.6	706
7	250	4.6	763
Residue	250	24.7	1160
Falling-film still. Soybean oil methyl esters (P.V. 1410). Pressure: 5–10 μ. Fractions recycled at each temperature.			
1	120	73.7	287
2	200	16.5	527
3	250	5.4	708
Residue	250	4.4	1350
"Rota-Film" still (Asco "50").[b] Methyl linoleate (P.V. 2210). Pressure 5–10 μ.			
1	120	65.9	300
2	200	28.1	603
Residue	200	6.0	1140

[a] Consolidated Vacuum Corporation, Rochester, N. Y.
[b] A. F. Smith Company, Rochester, N. Y.

Analytical Procedures. Molecular weights were determined cryoscopically in benzene saturated with water (to minimize absorption of moisture from the atmosphere). Peroxides were determined iodometrically by reduction with potassium iodide (12) or hydriodic acid (15). Iodine values were determined by the standard Wijs method. Attempts were made to hydrogenate the dimers in ethyl acetate solution with palladium catalyst at atmospheric pressure. However the dimers were not completely hydrogenated as indicated by the slow hydrogen absorption, which did not reach a maximum value in several hours. The difficulty in obtaining quantitative measurement of unsaturation of polymeric material is well known (35). Carbonyl and oxirane oxygen were determined by procedures similar to those described by Knight and Swern (21). Diene conjugation was determined spectrophotometrically in absolute methanol by using a Carey Model 14 instrument. The infrared measurements were made with a Baird-Atomic KM-1 instrument, using sodium chloride cells. An infrared procedure was devised for hydroxyl determination with methyl ricinoleate as standard. Beer's law was followed in the range of 1 to 5% ricinoleate. Dimers analyzed either in dilute carbon tetrachloride solutions (less than 5%) or undiluted gave results that deviated within 10% of the average and that agreed with the chemical analysis for hydroxyl groups (26).

Reaction with Hydrogen Iodide. Dimers (6 g., 0.01 M) were refluxed for 2–3 hrs. with constant boiling hydriodic acid (30 cc., 55–58%). The reaction mixture was extracted with ether, and the extract was treated with aqueous sodium bisulfite. Combined iodine was removed by refluxing in glacial acetic acid in the presence of zinc dust (35). After 30 min. the reaction mixture cleared, then it was filtered and extracted with ether. The extract was dried over sodium sulfate to give a yield of 94%. Since hydrogen iodide converted the dimers to the free acids they were remethylated with diazomethane prior to characterization.

Reduction Procedures

a) *Catalytic Hydrogenation.* Dimers were catalytically hydrogenated for 2 hrs. in a Parr shaker in ethyl acetate solution at 50–60°, 40 p.s.i. hydrogen pressure, with 3–6% platinum oxide catalyst.

b) *Sodium Sulfite.* The dimers (6 g.) were stirred with 20 ml. of ethanol and 5 g. of sodium sulfite for 24 hrs. The solution was filtered and extracted with ether. The extract was dried over sodium sulfate, and the solvent was removed *in vacuo.*

c) *Sodium Bisulfide.* Reduction with sodium bisulfite was carried out by the procedure of Knight and Swern (20) for the reduction of peroxides.

d) *Hydriodic Acid.* Reduction with hydriodic acid consisted of determining peroxide with hydriodic acid (18), followed by ether extraction of reduced dimers.

e) *Acetic Acid-Zinc.* The dimers (6 g.) were refluxed with 50 ml. glacial acetic and 5 g. zinc dust for 30 min. The mixture was poured in water and extracted with ether. The ether extract was washed with water, then with dilute alkali, followed by water until the washings were neutral, and was dried over sodium sulfate.

f) *Lithium Aluminum Hydride.* This (0.2 g., 0.005 mole) was suspended in 10 ml. of dry ether in a round-bottom flask and mixed with a magnetic stirrer in a nitrogen atmosphere. A solution of dimer (1.5 g., 0.0025 mole) was added dropwise to the suspension of lithium aluminum hydride from a dropping funnel stoppered with a drying tube. After addition of the dimer, the mixture was refluxed for 30 min. The lithium aluminum hydride was then inactivated with 25 ml. of 0.1 N hydrochloric acid. The reaction mixture was filtered, and the ether layer was washed with water and dilute acid, followed by water until the washings were neutral. The ether solution was dried over sodium sulfate, and the solvent was removed *in vacuo.*

Results

Polymerization of Autoxidized Fatty Esters and Fat Hydroperoxides. To determine the effect of hydroperoxide concentration on the extent of polymerization, a series of autoxidized fatty esters and of fat hydroperoxide mixtures were decomposed at 210°C. in nitrogen for 15 min. The decomposed esters were distilled molecularly at 120–140°/5 μ to remove the mixed monomeric materials. Results in Table II show a linear relation between the peroxide value of the esters before decomposition and the amount of polymer formed. There is good agreement between the experimental yields of polymers and the yields calculated on the basis that only hydroperoxides polymerize. In the presence of alkali-conjugated fatty esters, as with the nonconjugated fatty esters, the yields of polymers agreed with the yields calculated from the peroxide value of the mixtures. The fresh esters did not polymerize under conditions used for decomposition. Analyses of the monomeric fractions showed that they consist mainly of the nonoxidized methyl esters plus a small portion of low-molecular-weight decomposition products of hydroperoxides. In the monomeric fractions from the mixtures of conjugated methyl linoleate and methyl linoleate hydroperoxides, the diene conjugation increased in direct proportion to the concentration of conjugated methyl linoleate in the mixtures before heating (Figure 1).

TABLE II

Polymerization by Thermal Decomposition of Fatty Ester Hydroperoxides

Hydroperoxide mixtures	Peroxide value	Yield of polymer	
		Found	Calculated [a]
	me./kg.	%	%
Autoxidized soybean oil methyl esters	0	0	0.0
	113	2.6	1.8
	642	9.3	10.5
	1059	16	17.3
	1382	21	22.5
Mixtures of methyl linoleate hydroperoxides and methyl linoleate	0	0	0.0
	1325	25	21.6
	2650	43	43.3
	3975	65	64.9
	5300	78	86.5
Mixtures of methyl linoleate hydroperoxides and alkali-conjugated methyl linoleate	0	0	0.0
	1250	21	20.4
	2315	43	40.4
	3550	62	59.8
	4630	71	75.6

[a] Assuming that methyl linoleate hydroperoxide (P.V. 6125) gives 100% polymer.

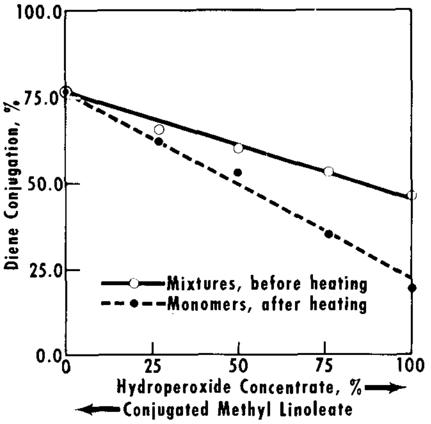


FIG. 1. Diene conjugation in mixtures of methyl linoleate hydroperoxide and alkali-conjugated methyl linoleate before and after heating.

The polymers from all the mixtures of hydroperoxides and methyl esters had approximately the same characteristics, *e.g.*, saponification equivalent, iodine values, and hydroxyl concentrations. The amount of volatiles produced from the polymerization of fat hydroperoxides in sealed tubes at 210°C. in inert atmosphere varied from 8 to 10% of the weight of hydroperoxides. These volatiles consisted of 48 to 52% water, which is equivalent to approximately one mole per mole of hydroperoxide.

The effect of autoxidation and subsequent thermal decomposition on the infrared spectra of soybean oil esters is shown in Figure 2. The autoxidized esters show an hydroxyl band at 2.95 μ and *cis-trans* conjugation at 10.2 and 10.6 μ, which are attributed to the formation of fat hydroperoxides (6,25,28,30). An accumulation of carbonyl compounds is also indicated by the broadening of the carbonyl band at 5.8 μ to 5.9 μ. On polymerization the hydroxyl band decreased and shifted from 2.95 to 2.88 μ. This absorption is attributed to a secondary hydroxyl group and is sub-

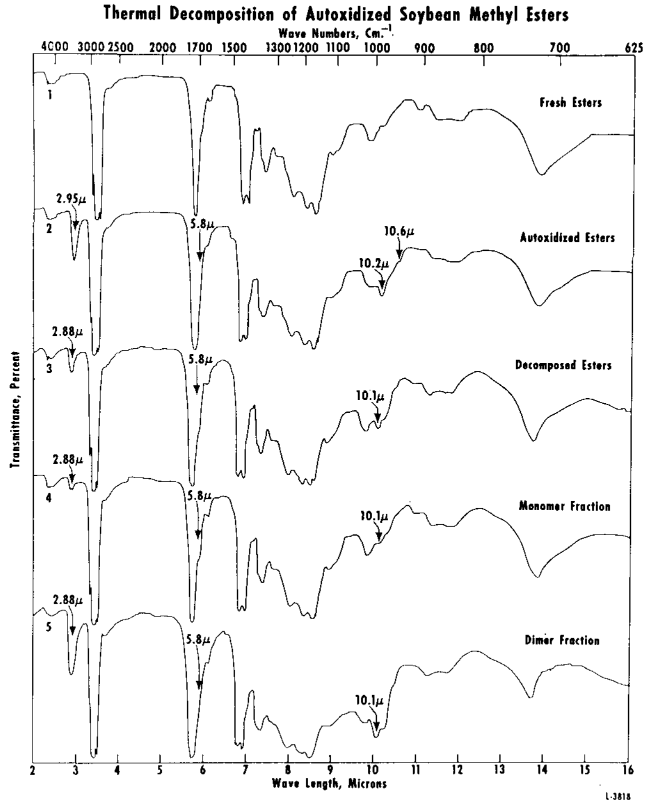


FIG. 2. Effect of thermal decomposition of autoxidized soybean methyl esters on infrared spectra.

stantiated by the absence in the polymerized esters of peroxides as determined iodometrically with potassium iodide. Only a trace of *cis-trans* absorption is shown in the autoxidized-thermally decomposed esters, which is replaced by *trans-trans* conjugation at 10.1 μ. The decrease in *cis-trans* conjugation was confirmed by the loss of diene conjugation measured at 232 mμ. After thermal decomposition the diene conjugation in the autoxidized esters decreased from 18.1% to 8.4% [using an absorptivity of 81.3 for pure methyl linoleate hydroperoxide (6)]. The broad carbonyl band at 5.8 μ remained unchanged in the decomposed esters. The monomer obtained by molecular distillation of the decomposed ester shows a much smaller hydroxyl band at 2.88 μ, less *trans-trans* absorption at 10.1 μ, and a narrower carbonyl band at 5.8–5.9 μ than the dimer. The diene conjugation measured at 232 mμ was 3.5% for the monomer and 23% for the dimer.

The infrared spectra of the polymeric fractions in thermally decomposed autoxidized safflower methyl esters were similar to each other (Figure 3).

Characterization of Dimers. The principal fractions obtained by molecular distillation of the various thermally decomposed hydroperoxides were the dimers. The yields of dimer in polymers from the decomposed hydroperoxides were safflower esters, 70; soybean esters, 62.7; soybean oil, 66.8; methyl oleate, 90.9; and methyl linoleate, 82.4%. The analyses of dimeric fractions are given in Table III. The unsaturation of the dimers can only be estimated from the iodine values measured, which probably represent minimum values (35). Determinations of hydrogen iodine values in the dimers agreed with those by the Wijs method, but precision was poor. Each dimer contains about two double bonds according to these data. The dimers from polyunsaturated fatty ester hydroperoxides showed diene conjugation ranging from 10 to 20%. This diene conjugation could be partly, but not entirely, attributed to conjugated carbonyl because a poor correlation exists between diene and the carbonyl content of the dimer. All the nonester oxygen cannot be accounted for by the hydroxyl and carbonyl oxygen.

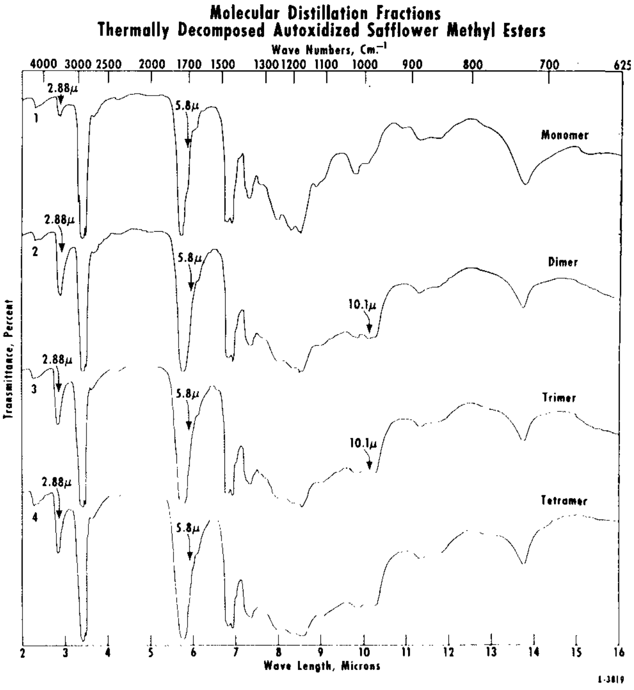


FIG. 3. Infrared spectra of molecular distillation fractions from thermally decomposed autoxidized safflower esters.

The dimers of polyunsaturated esters contain about 1 mole hydroxyl and 0.5 mole of carbonyl per mole of dimer. The concentration of these functional groups is somewhat lower in the oleate dimer.

In Figure 4 a comparison is made of the infrared spectra of methyl oleate and methyl linoleate with their corresponding hydroperoxide dimers. The spectra of the dimers were similar to those of the original fatty esters except for the appearance of a band at 2.85 μ for the secondary hydroxyl group, a broadening of the carbonyl band at 5.7–5.8 μ, and a decrease in the *cis* peak at 6.1 μ. The oleate dimer showed a

TABLE III

Characteristics of Thermal Dimers

Dimers	Mol. wt.	Sap. equiv.	C	H	O (by diff.)	Iodine value	Diene	OH	Carbonyl
			%	%	%		%	*mole/mole*	*mole/mole*
Methyl oleate	552	290	74.2	11.2	14.6	76.7	3.7	0.36	0.23
Methyl linoleate	603	304	73.6	10.4	16.0	98.0	23.4	0.64	0.52
Safflower methyl esters	544	263	70.9	11.5	18.6	83.3	29.5	1.1	0.55
Soybean methyl esters	527	299	72.9	10.6	16.5	96.2	23.0	1.0	0.34
Soybean oil	546	288	73.1	10.6	16.0	86.5	12.3	7.2	0.31
Dimeric oleate (calculated)	591	296	77.8	11.2	11.0	86.1	......	......	......

TABLE IV

Characteristics of Reduced Thermal Dimers

Dimers	Iodine value	Diene	OH	Mol. wt.	Infrared		
					Cis-trans	*Trans-trans*	Isol. *trans*
		%	%				
Soybean ester dimer	96.2	23.0	3.1	530	+	+	+
Hydrogenated dimer	22.2	0	3.1	540	—	—	+
Soybean oil dimer	......	12.3	3.5	......	+	+	+
Reduced dimer with:							
Sodium sulfite	......	11.1	3.6	......	+	+	+
Sodium bisulfite	......	10.9	3.7	......	+	+	+
Zinc-acetic acid	......	9.3	2.6	......	+	+	+
Hydriodic acid	......	6.5	free acid	......	—	—	+
Lithium aluminum hydride	......	1.1	5.9	......	—	—	—

sharp band for isolated *trans* at 10.3 μ which is the same as the corresponding oleate hydroperoxide (20), whereas the linoleate dimer showed absorption for *trans-trans* conjugation at 10.1 μ (16) and for *cis-trans* conjugation at 10.2 and 10.5 μ.

Since the oxygen unaccounted for in the dimers varied from 0.4 to 2%, the possible presence of other oxygen-containing groups was investigated. The dimers showed no oxirane oxygen or peroxidic oxygen iodometrically with potassium iodide. Evidence of other peroxidic oxygen was sought by using hydriodic acid. This reagent is used for determining ditertiary butyl peroxide (9), which is not reduced by potassium iodide (21). The dimer from soybean ester hydroperoxides was reduced with hydriodic acid, and the liberated iodine was equivalent to 2.65% "active" oxygen, a value higher than the oxygen unaccounted for (1.4%). On catalytic hydrogenation of the dimer the active oxygen content decreased from 2.65 to 0.4%. This evidence may possibly indicate the presence of intramolecular peroxide groups in the dimers.

Reaction of the soybean ester hydroperoxide dimer (OH, 3.1; diene, 23; oxygen, 16.5%; mol. wt., 530) with hydrogen iodide eliminated the hydroxyl group and diene conjugation. The hydrogen iodide-treated dimer had the following characteristics: OH, 0; diene, 0; oxygen, 13.0%; mol. wt., 670. Elimination of the hydroxyl group accounts for the decrease in oxygen content. Since no cleavage of the dimer occurred, it is concluded that a carbon-to-carbon bond exists between the monomeric units.

The reduction of soybean oil and soybean ester hydroperoxide dimers with different agents is shown in Table IV. Hydriodic acid and lithium aluminum hydride were the only chemical reducing-agents that decreased the diene conjugation. Lithium aluminum hydride almost doubled the hydroxyl content indicating the reduction of one ester group to the alcohol. Although the catalytic hydrogenation of the dimer was not complete, the diene conjugation was eliminated by this reaction. The molecular weight of the hydrogenated dimer did not change.

The attempted aromatization of methyl linoleate hydroperoxide dimer by bromination with N-bromosuccinimide and dehydrobromination with N,N-dimethyl aniline (32) failed. The dehydrobrominated dimer showed no aromatic absorption in the ultraviolet region. The residue (2.6%) obtained after exhaustive oxidation with permanganate (5 hrs.) was also not aromatic before or after sublimation. Since the presence of an hydroxyl group in the vicinity of a cyclic structure might prevent aromatization, the bromination-dehydrobromination procedure was repeated with the linoleate dimer, which was freed of hydroxyl by treating with hydrogen iodide and acetic acid-zinc. The products obtained after dehydrobromination and after permanganate oxidation (2%) showed no aromatic character in the ultraviolet.

The dimeric acids of methyl oleate and safflower ester hydroperoxides obtained by saponification of the dimer esters were subjected to permanganate-periodate oxidation (18) to estimate the position of double bonds. The titration per weight of acids obtained after oxidation was twice that of the starting dimeric acids. This effect would be expected from the formation of either two dibasic acids or one monobasic acid and a tribasic acid.

The oxidation treatment yielded a complex mixture of mono- and dibasic acids and other acids, which were not all identified. The chromatographic separations are shown in Table V. An appreciable polar fraction remained adsorbed on the column, which could be eluted with ether. The dibasic acid fractions were identified by chromatographing the mixed acids containing pure sebacic and azelaic acids. The presumed monobasic peaks were rechromatographed on a special column, using a stationary phase rich in methanol to separate C_{12} and C_{13} dibasic acids from pelargonic acid (17). The monobasic fraction from the safflower ester dimer was all recovered as a single peak showing no higher dibasic acids. It was rechromatographed on a Nijkamp column (24) and was identified to be chiefly pelargonic acid.

TABLE V

Chromatographic Separation of the Acids from Dimers after Oxidation with Permanganate-Periodate

Fractions	Oleate dimer	Safflower ester dimer
	%	%
1. "Monobasic" peak	41.4	26.6
2. C_{10} dibasic acid	0.0	6.6
3. C_9 dibasic acid	9.9	30.2
4. C_8 dibasic acid	15.1	10.4
5. C_7 dibasic acid	4.2	......
6. C_6 dibasic acid	5.6	......
7. Ether eluate	14.9	......
Total recovery	91.6	73.8

The presumed monobasic fraction from oleate dimer on rechromatographing yielded 70% of a monobasic peak and 21% of a mixture of polar dibasic acids which were not completely separated. The first peak (70%) gave only 27% monobasic steam-distillable acids. The nondistillable residue had a neutralization equivalent of 330, indicating that it consisted of the original dimeric acid which was not oxidized by the permanganate-periodate procedure. The complete fractionation of the acids from oxidized methyl oleate dimer gave the following composition: dibasic acids (C_6 to C_{10}), 35%; unidentified polar dibasic acids, 9%; polar acids eluted with ether, 15%; steam-volatile monobasic acids, 8%; and dimer not oxidized, 15%. It is concluded that the dimeric acids behave like monounsaturated mixtures containing double

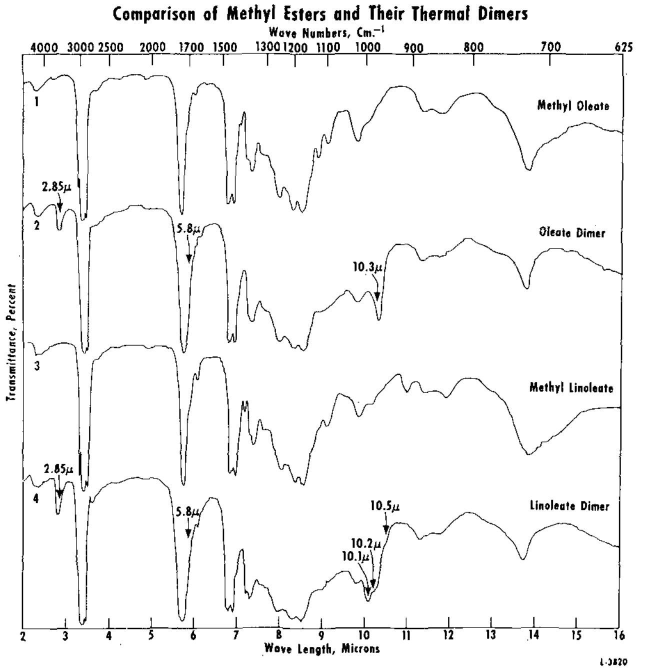


FIG. 4. Infrared spectra of methyl esters and the corresponding hydroperoxide dimers.

bonds in positions C_8, C_9, and C_{10} in the safflower ester dimer and in positions C_6, C_7, C_8, C_9, and C_{10} in the oleate dimer.

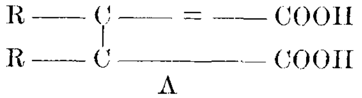
Discussion

The thermal decomposition of autoxidized fatty esters at 210°C. gave yields of polymers approximately equal to the percentage of concentration of hydroperoxides. The dilution of hydroperoxides with *alkali-conjugated methyl linoleate did not increase the* yields of polymers. Furthermore the characteristics of the polymers obtained from mixtures of hydroperoxides and nonconjugated and conjugated fatty esters were similar. These data suggest that only the hydroperoxides are involved in the polymerization reactions.

This conclusion is in contrast with the deduction of Williamson (35) that methyl linoleate and its hydroperoxide are involved in the polymerization. He carried out the decomposition at 100°C. to 90% completion and found that the oxygen content of the monomeric compounds formed was equal to more than half the oxygen present in the hydroperoxide. At the levels of autoxidation studies in the present paper, the hydroxyl content of the monomeric products was only a small part of that found in the polymers. Our dimers from polyunsaturated fatty ester hydroperoxides are similar to those isolated by Williamson since they showed a carbon-carbon bond between the monomeric units. However our dimers showed about half as much unsaturation and diene conjugation and more oxygen than the principal dimeric fraction isolated by Williamson. A smaller dimeric fraction isolated by him showed more similar characteristics (low unsaturation and higher oxygen) to the dimers isolated in the present work. Williamson postulated that this second dimeric product is derived from the first major dimer, probably through reaction with hydroxyl radicals.

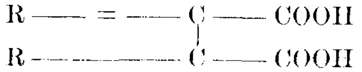
A number of analytical problems remain to be further explored before one can obtain a clearer picture of the structure of these hydroperoxide dimers. The oxygen content of the dimers, which was not all accounted for by the hydroxyl and carbonyl content, may only be a reflection of some inaccuracy in the analytical methods used. The data on hydriodic acid reduction suggest a source of additional oxygen in the dimers, which could be attributed to difficultly reducible peroxide linkages, *e.g.*, intramolecular peroxides. The presence of these linkages in various products of autoxidation and polymerization has been suggested by different workers (4,5,6,22). Such linkages, like the dialkyl peroxides, are not reducible with potassium or sodium iodide (29,33). Hydriodic acid reduction is a standard procedure to determine dialkyl peroxides like ditertiary butyl peroxide (9). Recent unpublished evidence in this laboratory indicates that the presence of allylic unsaturation in alcohols and corbonyl compounds, as well as epoxy groups, give a positive test for hydriodic reduction. This may account for the excessive oxygen values obtained by hydriodic acid reduction of the dimers.

The complex structure of the dimers is demonstrated by the mixtures of acids obtained with permanganate-periodate oxidation. One of the double bonds in the dimers was resistant to this oxidation treatment, possibly as a result of steric hindrance. The acids from oxidative fission of the oleate dimer support the following structures:



$$\begin{array}{l} R—C——=——COOH \\ \quad\ \ | \\ R—C————COOH \end{array}$$

A



$$\begin{array}{l} R——=——C—COOH \\ \qquad\qquad\ \ | \\ R————C—COOH \end{array}$$

B

Structure A on oxidative splitting would yield one short dibasic acid (C_6 to C_9) and a higher dibasic acid (C_{27} to C_{30}) whereas Structure B would yield one short monobasic acid and a higher tribasic acid. The isolation of only 8% monobasic acid from this dimer would indicate that Structure A is the predominant one. Although only the C_6 to C_{10} dibasic acids and the C_9 monobasic acid were positively identified, the presence of polar higher dibasic acids containing an hydroxyl group or a tribasic acid is probably indicated in the highly polar chromatographic fraction that was eluted with ether.

The establishment of a mechanism for the dimerization of fat hydroperoxides must await further kinetic investigations of this reaction. The present data however do not support a Diels-Alder addition since no evidence of a six-membered cyclic structure was obtained by bromination-dehydrobromination of the thermal dimers. This mechanism is excluded in the thermal decomposition of methyl oleate hydroperoxides that yielded 90% dimer. From present information on the decomposition of organic hydroperoxides (34) one may postulate that the thermal decomposition of fat hydroperoxides yields an alkoxy and a hydroxyl radical by homolytic fission of the hydroperoxide linkage. These radicals would, in turn, attack other hydroperoxide molecules to yield water and alkyl radicals that would either dimerize with themselves or with alkoxy hydroperoxide radicals. The failure of added alkali-conjugated fatty esters to react and the substantial loss of conjugation from the hydroperoxides during the reaction suggest that the hydroperoxide free radicals must rearrange before dimerization. Since the hydroperoxides are conjugated, their free radicals would be more reactive with each other than with the nonconjugated ester molecules (14). The high proportion of dimers obtained under our conditions of decomposition is therefore attributed to interaction of free radicals derived by homolytic fission of the hydroperoxides.

Summary

When autoxidized fatty esters and purified fatty hydroperoxides were decomposed in the absence of oxygen at 210°C., the principal reaction was dimerization of the fatty acid chains with elimination of the hydroperoxide groups. Dimers isolated by molecular distillation (60 to 90% of the polymer) have approximately 1 mole hydroxyl, 0.5 mole carbonyl, and two double bonds per mole of dimer. Diene conjugation in the dimers from polyunsaturated fat hydroperoxides varied from 10 to 23%. The infrared spectra of the dimers were similar to those of the original fatty esters except for one striking band at 2.9 μ, which is attributed to the secondary hydroxyl group. The *cis-trans* diene in the polyunsaturated hydroperoxides was isomerized to the *trans-trans* configuration on dimerization. The methyl oleate hydroperoxide dimer showed only absorption for isolated *trans* double bond. The dimer was not split either by catalytic hydrogenation or by hydrogen iodide, indicating a carbon-carbon bond between the monomer units. On oxidation with permanganate and periodate, the dimeric acids behaved like a monounsaturated mixture containing double bonds in the C_6, C_7, C_8, C_9, and C_{10} positions in the oleate dimer and in the C_8, C_9, and C_{10} positions in the safflower ester dimer. Although the dimers showed no peroxidic oxygen iodometrically with potassium iodide, a reduction occurred with hydriodic acid that may indicate the presence of intramolecular peroxide groups and/or allylic alcohol or carbonyl groups. Bromination with N-bromosuccinimide and dehydrobromination with N,N-dimethyl aniline produced no aromatization. Subsequent oxidation of the dehydrobrominated dimer yielded 2.6% residue, which was not aromatic. This evidence indicates that the dimer does not have a six-membered cyclic structure. Dimerization of the hydroperoxides is suggested as occurring through alkyl or alkoxy hydroperoxide radicals to give carbon-carbon linked fatty acid dimers and some higher polymeric units.

Acknowledgments

The authors wish to thank E. Selke for determination of ultraviolet, infrared spectra, and standardization of hydroxyl determinations; to E. P. Jones for invaluable help in the permanganate-periodate oxidations and in chromatographic separations; to R. E. Beal for supplying the linoleic acid; to C. R. Scholfield for the alkali-conjugated linoleic acid; and to Mrs. C. McGrew for the elemental analyses.

REFERENCES

1. Banks, A., Keay, J. N., and Smith, J. G. M., Nature *179*, 1078 (1957).
2. Beal, R. E., and Brekke, O. L., J. Am. Oil Chemists' Soc., *36*, 397 (1959).
3. Bergström, S., Nature, *156*, 717 (1945).
4. Bergström, S., Blomstrand, R., and Laurell, S., Acta Chem. Scand., *4*, 245–250 (1950).
5. Bolland, J. L., and Gee, G., Trans. Faraday Soc., *42*, 236, 244 (1946).
6. Cannon, J. A., Zilch, K. T., Burket, S. C., and Dutton, H. J., J. Am. Oil Chemists' Soc., *29*, 447 (1952).
7. Chang, S. S., and Kummerow, F. A., J. Am. Oil Chemists' Soc., *30*, 403 (1953).
8. Cowan, J. C., and Carter, H. E., Lipids in "Organic Chemistry," Ed. H. Gilman, Vol. III, p. 193, New York, John Wiley and Sons, 1953.
9. Dickey, F. H., Raley, J. H., Rust, F. F., Treseder, R. S., and Vaughan, W. E., Ind. Eng. Chem., *41*, 1673 (1949).
10. Ellis, G. W., Biochem. J., *46*, 129 (1950).
11. Evans, C. D., Frankel, E. N., Cooney, P. M., and Moser, M. A., J. Am. Oil Chemists' Soc., *37*, 452–456 (1960).
12. Fugger, J., Cannon, J. A., Zilch, K. T., and Dutton, H. J., J. Am. Oil Chemists' Soc., *28*, 285 (1951).
13. Gaddis, A. M., Ellis, R., and Currie, G. T., Food Res., *24*, 283 (1959).
14. Gray, P., and Williams, A., Trans. Faraday Soc., *55*, 760–777 (1959).
15. Handy, C. T., and Rothrock, M. S., J. Am. Chem. Soc., *80*, 5306 (1958).
16. Jackson, J. E., Paschke, R. F., Tolberg, W. E., Boyd, M. M., and Wheeler, D. J., J. Am. Oil Chemists' Soc., *19*, 229 (1952).
17. Jones, E. P., unpublished results.
18. Jones, E. P., and Stolp, J. A., J. Am. Oil Chemists' Soc., *35*, 71–76 (1958).
19. Kawahara, F. K., and Dutton, H. J., J. Am. Oil Chemists' Soc., *29*, 372 (1952).
20. Knight, H. B., Eddy, C. R., and Swern, Daniel, J. Am. Oil Chemists' Soc., *28*, 188 (1951).
21. Knight, H. B., and Swern, Daniel, J. Am. Oil Chemists' Soc., *26*, 366 (1949).
22. Lundberg, W. O., Chipault, J. R., and Hendrickson, M. J., J. Am. Oil Chemists' Soc., *26*, 109 (1949).
23. Morris, S. G., J. Agr. Food Chem., *2*, 126 (1954).
24. Nijkamp, H. J., Anal. Chim, Acta., *5*, 325 (1951).
25. O'Connor, R. T., J. Am. Oil Chemists' Soc., *31*, 1 (1956).
26. Ogg, C. L., Porter, W. L., and Willits, C. D., Ind. Eng. Chem., Anal. Ed., *17*, 394 (1945).
27. Powers, P. O., Ind. Eng. Chem., *41*, 304 (1949).
28. Privett, O. S., Lundberg, W. O., Khan, N. A., Tolberg, W. E., and Wheeler, D. H., J. Am. Oil Chemists' Soc., *30*, 61 (1953).
29. Raley, J. M., Rust, F. F., and Vaughan, W. E., J. Am. Chem. Soc., *70*, 1336 (1948).
30. Shreve, O. D., Heether, M. R., Knight, H. B., and Swern, Daniel, Anal. Chem., *23*, 277 (1951).
31. Swern, Daniel, Knight, H. B., Scanlan, J. T., and Ault, W. C., J. Am. Chem. Soc., *67*, 1132 (1945).
32. Teeter, H. M., Bell, E. W., Danzig, M. J., J. Am. Chem. Soc., *23*, 1156 (1958).
33. Wagner, C. D., Smith, R. H., Peters, E. D., Ind. Eng. Chem., Anal. Ed., *19*, 976 (1947).
34. Walling, C., "Free Radicals in Solution," p. 503, New York, John Wiley and Sons, 1957.
35. Williamson, L., J. Appl. Chem., *3*, 301 (1953).
36. Witting, L. A., Chang, S. S., and Kummerow, F. A., J. Am. Oil Chemists' Soc., *34*, 470 (1957).
37. Zilch, K. T., Dutton, H. J., and Cowan, J. C., J. Am. Oil Chemists' Soc., *29*, 244 (1952).

[Received February 12, 1960]