THE OXIDATION OF UNSATURATED FATTY ACIDS

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This article is compiled from several papers in Japanese by the author and his coworkers, of which one will be published shortly and the others have already been published, and is concerned with the relative rate of oxidation of isomeric monoethylenic acids, the relative rate of oxidation of monoethylenic and polyethylenic acids, and the course of oxidation of polyethylenic acids. Also the results of an examination of oleic acid and its esters autoxidized in the course of long storage are recorded.

I. The Rates of Oxidation of 9-, 12-, and 15-Octadecenoic Acids with Molecular Oxygen and with Perbenzoic Acid[1]

As is the case with hydrogenation and halogen addition, the rate of oxidation of an unsaturated fatty acid depends upon the position of its respective ethylenic linkage. But adequate information on the relation between the position of ethylenic linkage and the susceptibility to oxidation appears to be lacking. Holm, Greenbank, and Deysher[2] found that oleic acid absorbs oxygen more rapidly than ricinoleic acid which has an ethylenic linkage in the same position as oleic acid and a hydroxyl group at the 12-carbon. The work of Kuhn and Meyer,[3] who compared the rates of oxygen absorption of unsaturated fatty acids and their esters in the presence of hemin, indicated that oleic acid oxidizes 50 times more easily than its geometrical isomer, elaidic acid, while 9-decenoic and 10-undecenoic acids, both having ethylenic linkage at the end of carbon chain, are rather stable. Olive oil, which consists substantially of the glycerides of oleic acid, oxidizes at 1/2, and ethyl oleate at 1/8 the velocity of oleic acid. Täufel and his coworkers[4] concluded from the results of the determinations of weight increase and rancidity in the course of air oxidation of oleic, elaidic, erucic, and brassidic acids that oleic acid oxidizes most easily, erucic acid oxidizes much more slowly than oleic acid, and elaidic and brassidic acids are stable. Comparing the rates of oxygen absorption of oleic and elaidic acids and their methyl esters, Miyoshi and Ibuki[5] found that the cis-isomers, both fatty acid and methyl ester, absorb oxygen more rapidly than the corresponding trans-isomers, and also that the rate of oxygen absorption of fatty acid is greater than that of its methyl ester.

The above literature reveals that the susceptibility of a monoethylenic acid, such as octadecenoic acid, to oxidation is affected not only by the position of ethylenic linkage but also by the spatial configuration and the other functional groups of that acid. With the purpose of studying the relation between the rate of oxidation and the position of ethylenic linkage as well as the spatial configuration in the case of octadecenoic acids, 9-(oleic and elaidic), 12- and 15-octadecenoic acids have been prepared, and the rates of oxidation of these acids with molecular oxygen in acetic

acid solution in the presence of cobalt resinate as a catalyzer and also with perbenzoic acid in chloroform have been determined. The position of ethylenic linkage in these octadecenoic acids corresponds to the position of each ethylenic linkage in linoleic (9, 12-octadecadienoic) and linolenic (9, 12, 15-octadecatrienoic) acids. The results of these experiments show that the rates of oxygen absorption in acetic acid solution are nearly the same for each of the isomers, while the rates of oxidation of 9-octadecenoic (oleic and elaidic) acids with perbenzoic acid are greater than those of 12- and 15-octadecenoic acids.

1. Preparation of four isomerides of octadecenoic acid. Oleic acid (*cis*-9-octadecenoic acid):—Mixed fatty acids of Japanese camellia oil were treated by the lead salt alcohol method in order to remove the solid fatty acids, the liquid fatty acids were converted into the lithium salts, and the latter were treated with aqueous ethanol (50%). The lithium salts crystallized out from aqueous ethanol were separated and acidified with hydrochloric acid. The fatty acids thus liberated were esterified with methanol, and the methyl esters were fractionally distilled. Oleic acid was obtained from a methyl ester fraction boiling at about 200°C/10 mm. d_4^{20} 0.8949, n_D^{20} 1.4595, N.V. 199.2, I.V. (Wijs) 90.3 (calculated for $C_{18}H_{34}O_2$: N.V. 198.6, I.V. 89.8).

Elaidic acid (*trans*-9-octadecenoic acid):—Oleic acid obtained above was elaidinized by means of nitrous acid. The product, after purification, had M.P. 43.5–44°C and I.V. 90.1.

12-Octadecenoic acid:—Following the method of Grün and Czerny,[6] fatty acids of hydrogenated castor oil having M.P. 76.5–77°C, S.V. 180.5, I.V. 3.9 and Acetyl V. 109.5 were converted into the ethyl esters, and the latter were recrystallized three times from 90% ethanol giving ethyl 12-hydroxystearate of M.P. 51–51.5°C, S.V. 170.5 (calculated, 170.9) and Acetyl V. 151.0 (calculated, 152.0). This was dehydrated by heating at 200°C for one hour in the presence of 2% of β-naphthalene sulfonic acid, the product was reconverted into the ethyl ester, and the latter was fractionated giving a fraction of B.P. about 180°C/2 mm, S.V. 178.5 and I.V. 79.6 (calculated for ethyl 12-octadecenoate: S.V. 180.8, I.V. 81.8). Since the free acid obtained from this fraction was contaminated with a crystalline solid, it was dissolved in petroleum ether, the solution was cooled to −5°C, and the crystalline deposit was removed by filtration. The liquid acid obtained from the filtrate appeared to consist chiefly of *cis*-isomer; d_4^{20} 0.8952, n_D^{20} 1.4599, N.V. 199.2, I.V. 87.8.

15-Octadecenoic acid[7]:—Starting from ethyl erucate, ω-chlorotridecanal was prepared, and the latter was converted into 13-hexadecenoic acid by the method of Noller and Bannerot,[8] from which 15-octadecenoic acid was obtained by malonic ester synthesis. The yield was very low. Since solid matter was rejected in the course of the purification of 15-octadecenoic acid, the final product seemed to consist mainly of *cis*-form; d_4^{20} 0.8961, n_D^{20} 1.4605, N.V. 197.6, I.V. 87.5.

2. Oxidation with molecular oxygen. To 0.5000 g of each sample of octadecenoic acids were added 0.0050 g of cobalt resinate and 10 cc of glacial acetic acid, and a 150-cc round bottom flask containing the above solution was placed on an eccentric shaking apparatus. The mouth of the flask was connected with a gas burette by a rubber tube. Prior to the experiments, the air in flask and gas burette was replaced by oxygen, and the water head of gas burette was kept at a fixed level. The experiments with each sample were carried out at the same time at room temperature of about 30°C, and the volume of oxygen absorbed was measured at an interval of

every 10 hours throughout 80 hours' experiments. A blank experiment without octadecenoic acid showed an oxygen absorption of less than 0.5 cc in the course of 40 hours. The results of the experiments are given in Table 1.

TABLE 1. Oxygen Absorption (cc, Reduced to 30°C, 1 atm.)

Time (hr)	10	20	30	40	50	60	70	80
Oleic acid	0.5	2.3	5.1	7.0	9.5	11.2	12.3	15.1
Elaidic acid	0.4	2.3	5.0	6.8	9.3	11.0	12.2	15.0
12-Octadecenoic acid	0.4	2.1	4.7	6.5	9.0	10.8	11.8	14.7
15-Octadecenoic acid	0.4	2.0	4.6	6.5	8.9	10.6	11.5	14.5

Assuming that one molecule of oxygen is quantitatively absorbed per one ethylenic linkage, the oxygen absorption calculated for 0.5000 g of octadecenoic acid would be 44.0 cc (30°C, 1 atm.). It is seen from Table 1 that four isomerides of octadecenoic acid have approximately the same rate of oxygen absorption. Neither *cis* and *trans* isomerides, such as oleic and elaidic acids, nor the position of ethylenic linkage affect materially the rate of oxygen absorption. These results do not comform to those obtained by previous authors. But this discordance is explicable if it is taken into consideration that the relative rate of oxidation is markedly affected by the experimental conditions. While the present experiments were carried out in acetic acid solution, the experiments of Täufel and his coworkers, for instance, were carried out with liquid oleic acid and crystalline elaidic acid. The fact that crystalline elaidic acid is much more stable against autoxidation than liquid oleic acid has also been verified by the present author who observed that while elaidic acid remained unaltered after long storage, oleic acid and its methyl and ethyl esters kept in the same place for the same period underwent autoxidation to a marked extent.

3. Oxidation with perbenzoic acid. A solution of perbenzoic acid in chloroform having 0.15% of active oxygen was prepared. Fifty cc of this solution contains approximately twice the quantity of perbenzoic acid equivalent to 1.0000 g of octadecenoic acid. Each sample (1.0000 g) of octadecenoic acids was weighed into a well-stoppered bottle, and 50 cc of the above solution of perbenzoic acid in chloroform was added. Five cc of the solution was periodically pipetted into another bottle which contained 10 cc of 5% solution of potassium iodide and 1 cc of 1 N hydrochloric acid, and the iodine liberated was titrated with N/10 sodium thiosulfate solution. Blank test was carried out corresponding to each experiment. From the difference in titres between the blank and the experiment with sample, the quantity of iodine equivalent to the oxygen absorbed by the sample was determined. In Table 2, oxygen absorption was expressed in terms of iodine value, *i.e.*, equivalent percentage absorption of

TABLE 2. Oxygen Absorption with Perbenzoic Acid, Expressed in Terms of Iodine Value

	Time (min.)	15	30	45	60	90	150	360	1440
A	Oleic acid	52.3	68.5	75.5	80.5	83.7	87.8	89.8	90.5
A	12-Octadecenoic acid	30.5	47.5	55.3	60.7	64.5	70.2	74.5	86.7
A	15-Octadecenoic acid	28.7	45.0	54.0	57.8	62.6	69.5	73.2	86.0
B	Oleic acid	53.1	69.6	76.5	81.3	84.5	89.0	89.8	90.3
B	Elaidic acid	51.2	67.1	75.3	80.7	84.0	88.9	89.5	90.0

iodine. Experiments A and B were performed at room temperatures of 22–24.5°C and 23–24°C, respectively.

Table 2 indicates that oleic acid absorbs oxygen faster than 12- and 15- octadecenoic acids. The rates of oxidation of oleic and elaidic acids show no significant difference, although oleic acid seems to absorb oxygen at a slightly faster rate. Likewise, the rates of oxidation of 12- and 15-octadecenoic acids show no significant difference, although the rate of oxidation of 12-octadecenoic acid seems to be but slightly greater. It should be noted that since the iodine values (Wijs) of each sample of octadecenoic acid used in these experiments are 90.3, 90.1, 87.8, and 87.5, respectively, for oleic, elaidic, 12-octadecenoic, and 15-octadecenoic acids, the unsaturations of oleic and elaidic acids are a little higher than those of 12- and 15-octadecenoic acids. As the possibility is not excluded that dissimilarity of unsaturation of each sample affects the rate of oxidation, each sample of 12- and 15-octadecenoic acids was added with a small amount of linoleic acid so as to make the iodine values 90.0 and 90.5, respectively, for 12- and 15-octadecenoic acids; then another series of experiments on the oxidation with perbenzoic acid was carried out at room temperature of 25–26.5°C using these samples of the same unsaturation. In Table 3 are presented the results of these experiments, which are quite consistent with the results recorded in Table 2, indicating that oleic acid absorbs oxygen more rapidly than 12- and 15-octadecenoic acids. The latter two acids show little difference in the rate of oxygen absorption.

TABLE 3. Oxygen Absorption with Perbenzoic Acid, Expressed in Terms of Iodine Value

Time (min.)	15	30	45	60	90	150	360	1440
Oleic acid	55.0	71.2	78.2	82.0	85.5	89.2	89.8	90.2
12-Octadecenoic acid	46.0	58.3	66.5	71.6	76.0	81.5	84.2	89.7
15-Octadecenoic acid	45.5	56.5	64.3	69.8	74.5	80.8	84.0	89.9

II. The Rates of Oxidation of Oleic, Linoleic, and Linolenic Acids with Molecular Oxygen and with Perbenzoic Acid[9]

Concerning the relation between the rate of oxidation and the degree of unsaturation of unsaturated fatty acids, Kuhn and Meyer[10] found by comparing the rates of oxidation of ethyl oleate, methyl linoleate, olive oil, and linseed oil in the presence of hemin that the increase from 1 to 2 ethylenic linkages multiplies the oxidation velocity by 12, while the increase from 1 to 3 ethylenic linkages multiplies it by 100. Also Täufel and Suess[11] found that the susceptibility of linoleic acid to autoxidation is disproportionately large as compared to that of oleic acid when the number of ethylenic linkage is considered. On the other hand, Stirton, Turner and Riemenschneider[12] measured the oxygen absorption of methyl linolenate, linoleate and stearate, and methyl ester of lard with the results that mixtures of methyl esters absorbed oxygen at a rate which could be approximately predicted from the rate of oxygen absorption of each component and the percentage of each present. Hilditch and Sleightholme,[13] however, indicated that the rate of oxidation of oleate is accelerated by the presence of a small amount of linoleate.

The author has examined in the present study the rates of oxidation of oleic, linoleic, and linolenic acids under similar conditions as described in the preceding experiments with four isomerides of octadecenoic acid.

1. Oxidation with molecular oxygen. The sample of oleic acid used in these experiments was the same as that used in the preceding experiments. Linoleic and linolenic acids were obtained by saponifying methyl linoleate and linolenate which were used in the subsequent experiments to be described later. Iodine values (Wijs) were found to be 90.3, 181.0, and 271.5, respectively, for oleic, linoleic and linolenic acids. Calculated values for three acids are 89.8, 181.0, and 273.5 respectively. Each sample (0.5000 g) was dissolved in 10 cc of glacial acetic acid to which was added 0.0050 g of cobalt resinate, and the solution was exposed to oxygen in a similar way as described in the preceding experiments at room temperature of about 30°C. The rates of oxygen absorption are given in Table 4, from which it is seen that linolenic acid absorbs oxygen at a faster rate than linoleic acid, which in turn absorbs oxygen at a faster rate than oleic acid. If one molecule of oxygen is assumed to be absorbed quantitatively by one ethylenic linkage, the oxygen absorptions (30°C, 1 atm.) calculated for 0.5000 g of each sample would be 44.0 cc for oleic acid, 88.7 cc for linoleic acid, and 134.0 cc for linolenic acid.

TABLE 4. Oxygen Absorption (cc)

Time (hr)	10	20	30	40	50	60	70	80
Oleic acid	0.5	2.3	5.1	7.0	9.5	11.2	12.3	15.1
Linoleic acid	4.5	15.2	30.1	38.6	45.0	52.0	58.1	62.5
Linolenic acid	7.7	30.5	60.5	72.0	83.4	93.5	100.2	105.4

Table 5 shows the oxygen absorption expressed in percentage of the calculated value, *i.e.*, the average rate of oxygen absorption per one ethylenic linkage. It is largest for linolenic acid, followed by linoleic acid, and is far smaller for oleic acid.

TABLE 5. Oxygen Absorption (%, Based on the Quantitative Absorption)

Time (hr)	10	20	30	40	50	60	70	80
Oleic acid	1.1	5.2	11.6	15.9	21.6	25.4	27.9	34.3
Linoleic acid	5.1	17.1	33.9	43.5	50.7	58.6	65.5	70.5
Linolenic acid	5.7	22.8	45.1	53.7	62.2	69.8	74.8	78.7

Iodine value of each sample after 80 hours' oxidation was determined by the Wijs method, and also the iodine which was liberated from potassium iodide due to the presence of peroxide was determined with each sample after 80 hours' oxdation. Assuming that peroxide liberates iodine quantitatively from potassium iodide, the true iodine value corresponding to the ethylenic linkage after 80 hours' oxidation is obtainable as the amount of iodine liberated due to the presence of peroxide, expressed in percentage, plus the apparent iodine value determined in the usual way. From this true iodine value of the sample before and after oxidation, after correcting for the increase of molecular weight due to oxygen absorption, the ethylenic linkage which disappeared due to oxidation was calculated. The data are listed in Table 6.

If oxygen absorption takes place exclusively in the form of oxygen addition to ethylenic linkage, the corresponding figures in the last two columns in Table 6, should agree with each other. But the figures in the last column are larger than the corresponding figures in the preceding column, the difference being most marked for oleic acid and least marked for linolenic acid. This is attributable to other reactions than

TABLE 6. Loss of Ethylenic Linkage Due to Oxygen Absorption

	I.V. of original sample	I.V. after oxygen absorption	Iodine liberated by peroxide (%)	True I.V. after oxygen absorption	Loss of ethylenic linkage due to oxygen absorption (%)	Oxygen absorption (%, based on the calc'd value)
Oleic acid	90.3	62.3	15.0	77.3	11.2	34.3
Linoleic acid	181.0	46.5	22.5	69.0	58.8	70.5
Linolenic acid	271.5	42.5	29.5	72.0	71.0	78.7

oxygen addition to ethylenic linkage, among which the formation of hydroperoxide at α-methylenic carbon and the conjugation in the case of linoleic and linolenic acids are conceivable. The Wijs method does not give a correct iodine value for conjugated ethylenic linkages.

2. Oxidation with perbenzoic acid. Experiments were carried out at room temperature of 30–31.5°C in the same way as described in the preceding experiments with octadecenoic acids, except that the weights of samples taken were 1.0000 g, 0.5000 g, and 0.3333 g, respectively, for oleic, linoleic, and linolenic acids in order to maintain the same excess of perbenzoic acid for three acids. The results are given in Tables 7 and 8.

TABLE 7. Oxygen Absorption with Perbenzoic Acid, Expressed in Terms of Iodine Value

Time (min.)	15	30	45	60	90	150	360	1440	2880
Oleic acid	57.2	73.2	80.5	83.7	86.5	89.8	90.1	—	—
Linoleic acid	68.5	83.5	94.0	99.4	108.5	119.4	130.8	163.2	180.1
Linolenic acid	98.6	139.7	148.0	159.5	170.5	194.4	215.0	238.2	269.5

TABLE 8. Oxygen Absorption with Perbenzoic Acid (%, Based on the Calculated Value)

Time (min.)	15	30	45	60	90	150	360	1440	2880
Oleic acid	63.3	81.1	89.1	92.7	95.8	99.4	99.8	—	—
Linoleic acid	37.8	46.1	51.9	54.9	59.9	66.0	72.3	90.2	99.5
Linolenic acid	36.3	51.5	54.6	58.7	62.8	71.6	79.2	87.7	99.3

As is seen from Tables 7 and 8, the oxygen absorption after the same reaction period decreases on passing from linolenic to linoleic to oleic acid, while the oxygen absorption expressed in percentage against the calculated value is largest for oleic acid and considerably smaller for linoleic and linolenic acids. Accordingly the average rate of oxygen absorption per one ethylenic linkage of linoleic and linolenic acids is smaller than that of oleic acid. This is not in accordance with the oxidation with molecular oxygen in acetic acid solution, and it is suggested that the mechanism and course of oxidation with molecular oxygen in acetic acid differs from those of oxidation with perbenzoic acid.

If the oxidation with perbenzoic acid be assumed to be a bimolecular reaction, the velocity constant k will be found from the following equation:

$$k = \frac{\log a + \log (a_0 - a_\infty) - \log a_0 - \log (a - a_\infty)}{0.4343 \times a_\infty \times t}$$

where a, a_0, and a_∞ are cc of sodium thiosulfate solution required to titrate iodine liberated by perbenzoic acid remaining after respective reaction period. The results are recorded in Table 9.

TABLE 9. Velocity Constant of Oxidation with Perbenzoic Acid

Time (min.)		0	15	30	45	60	90	150	∞
Oleic acid	a	14.05	9.68	8.45	7.90	7.65	7.44	7.18	7.15
	$k \times 10^3$	—	5.9	5.4	5.1	4.7	4.5	4.0	—
Linoleic acid	a	14.05	11.41	10.82	10.42	10.21	9.87	9.43	7.09
	$k \times 10^3$	—	2.5	1.8	1.4	1.1	0.88	0.65	—
Linolenic acid	a	14.05	11.51	10.45	10.23	9.94	9.66	9.04	7.11
	$k \times 10^3$	—	2.4	2.0	1.5	1.3	0.98	0.78	—

While the velocity constant for oleic acid which has only one ethylenic linkage is found to be $k \times 10^3 = 5.9-4.0$, the velocity constant for linoleic and linolenic acids which have more than one ethylenic linkage do not lie within a narrow limit and become exceedingly small as the oxidation proceeds. This seems to point out that the reactivities of each ethylenic linkage of linoleic and linolenic acids are not alike and that they will change appreciably in the course of oxidation.

III. The Course of Oxidation of Methyl Linoleate[14]

It has been believed for many years that one molecule of oxygen adds to one ethylenic linkage at the first stage of autoxidation of unsaturated fatty acids or their esters, resulting in the formation of cyclic peroxide: $-CH=CH- + O_2 \longrightarrow -\underset{\diagdown\,\diagup}{\overset{O_2}{CH}}-CH-$.

However, Farmer[15] proposed the hydroperoxide theory which recognizes hydroperoxide $-\overset{OOH}{\overset{|}{C}}HCH=CH-$ as the main product at the initial stage of autoxidation, and the mechanism of the formation of hydroperoxide is explained as follows:

$$-CH_2CH=CH- + O_2 \longrightarrow CH_2\overset{*}{C}H-\overset{OO^*}{\overset{|}{C}}H-$$

$$-CH_2\overset{*}{C}H-\overset{OO^*}{\overset{|}{C}}H- + -CH_2CH=CH- \longrightarrow -CH_2\overset{*}{C}H-\overset{OOH}{\overset{|}{C}}H- + -\overset{*}{C}HCH=CH-$$

$$\longrightarrow -\overset{*}{C}HCH=CH- + O_2 \longrightarrow -\overset{OO^*}{\overset{|}{C}}HCH=CH-$$

$$-\overset{OO^*}{\overset{|}{C}}HCH=CH- + -CH_2CH=CH- \longrightarrow -\overset{OOH}{\overset{|}{C}}HCH=CH- + -\overset{*}{C}HCH=CH-$$

Also the radical $-CH_2\overset{*}{C}H-\overset{OOH}{\overset{|}{C}}H-$ participates in the formation of $-\overset{*}{C}HCH=CH-$. Thus the autoxidation proceeds in such a way that the carbon adjacent to ethylenic linkage or the α-methylenic carbon becomes the active center, and the hydroperoxide is formed by a chain reaction of the free radical $-\overset{*}{C}HCH=CH-$. While the hydro-

peroxide has not yet been separated from the oxidation products of linoleic and linolenic acids, the isolation of 8- and 11-hydroperoxides from the oxidized methyl oleate has already been reported.[16] However, it appears to be inadmissible to take no account of the formation of cyclic peroxide. The fall of iodine value in the course of autoxidation can not explained if hydroperoxide alone is formed exclusively. In short, it appears that even if hydroperoxide is formed firstly at the initial stage of autoxidation, it will be partly rearranged to cyclic peroxide, and the proportion of hydroperoxide and cyclic peroxide depends upon the conditions under which autoxidation takes place.[17] Another important feature found recently in the autoxidation of polyethylenic acids is the migration of ethylenic linkage with the formation of conjugated ethylenic linkages.[18] Caused by this conjugation, the position of carbon atoms which participate in the formation of peroxide becomes different from the position in the original polyethylenic acids. As for the susceptibility of respective ethylenic linkage of a polyethylenic acid to oxidation, the one which lies most remote from the carboxyl group, 12-ethylenic linkage of linoleic acid and 15-ethylenic linkage of linolenic acid, for instance, had been tacitly deemed most reactive; but Treubs[19] concluded from his study with methyl linoleate and linolenate that oxygen adds firstly to the ethylenic linkage nearest to the carboxyl group resulting in the formation of peroxide. Bergström[20] examined the oxidation product of peroxide type formed by oxidizing methyl linoleate with oxygen in the presence of inorganic catalyzer at 37°C with the results that it had conjugated ethylenic linkages and yielded, on hydrogenation, methyl esters of 9- and 13-hydroxystearic acids, from which the original peroxides were believed to consist of 9-hydroperoxide having 10, 12-ethylenic linkages and 13-hydroperoxide having 9, 11-ethylenic linkages.

The present study has been undertaken to know to which ethylenic linkage oxygen is added at the early stage of oxidation of methyl linoleate. For this purpose, methyl linoleate was dissolved in glacial acetic acid to which was added cobalt resinate as a catalyzer, and the solution was exposed to an atmosphere of oxygen at room temperature. When the oxygen absorption proceeded approximately to one mole per mole ester, the oxidation product was taken out and brominated, by which the unoxidized ethylenic linkage was saturated with bromine and thus protected against the oxidative scission in subsequent procedure. The brominated product was then subjected to oxidative scission with potassium permanganate, the scission product was debrominated, and the debrominated product was separated into several fractions. Each fraction was then examined.

1. Preparation of methyl linoleate. The mixed fatty acids of corn oil were brominated in ether solution under cooling at −5°C to −10°C, and the brominated product, after removal of excess of bromine and distilling off ether, was recrystallized twice from petroleum ether by which tetrabromostearic acid of M.P. 113–113.5°C was obtained in a yield of 59.0%. Also the mixed fatty acids of sesame oil were treated in the same way, by which tetrabromostearic acid was obtained in a yield of 33.3%. Tetrabromostearic acid obtained from both oils was debrominated with zinc and sulfuric acid in methanol giving methyl linoleate of S.V. 193.4 and I.V. 172.8 (calculated, S.V. 191.2, I.V. 172.4).

2. Oxidation of methyl linoleate. Twenty g of methyl linoleate was dissolved in 200 cc of glacial acetic acid to which was added 0.25 g of cobalt resinate as a catalyzer. The flask containing the solution was placed on a shaking machine and exposed to

an atmosphere of oxygen under a pressure of 20–30 cm water head at room temperature of 22–24.5°C. It required 13 hours and 30 minutes to absorb 1.5 l of oxygen, which is approximately the same with the calculated value, 1.521 l (0°C, 760 mm), corresponding to the absorption of one mole of oxygen per mole methyl ester. After this oxygen absorption, the solution was added with water, the oxidation product was extracted with a large quantity of ether, and after distilling off the ether, there was obtained an oily substance having S.V. 301.5, A.V. 32.0, I.V. 77.4, Acetyl V. 109.6 and Peroxide V. 40.0. Iodine value and peroxide value calculated for monoperoxide of methyl linoleate are 77.7 and 3063, respectively. Peroxide value found is very small as compared with the calculated value. Accordingly, the greater part of peroxide, if once formed, should have changed into other compounds under the conditions of oxidation in these experiments. There is a possibility, of course, that peroxide underwent decomposition in the course of the procedures taken after oxygen absorption.

3. Bromination of oxidation product, oxidative scission of brominated product with potassium permanganate, and debromination of scission product. Eighteen g of oxidation product was brominated in ether solution. Twenty-five g of brominated product was dissolved in 200 cc of acetone, 100 g of powdered potassium permanganate was added in small portions, and the mixture was refluxed for 10 hours to complete the reaction. The mixture was then added with water, sulphur dioxide was passed through it in order to reduce the oxides of manganese and the excess of potassium permanganate, and the scission product was extracted with a large quantity of ether. The scission product (25.5 g) was debrominated with zinc and sulfuric acid in methanol, and there was obtained 20.4 g of debrominated product having S.V. 402.9, I.V. 23.7 and Acetyl V. 79.1.

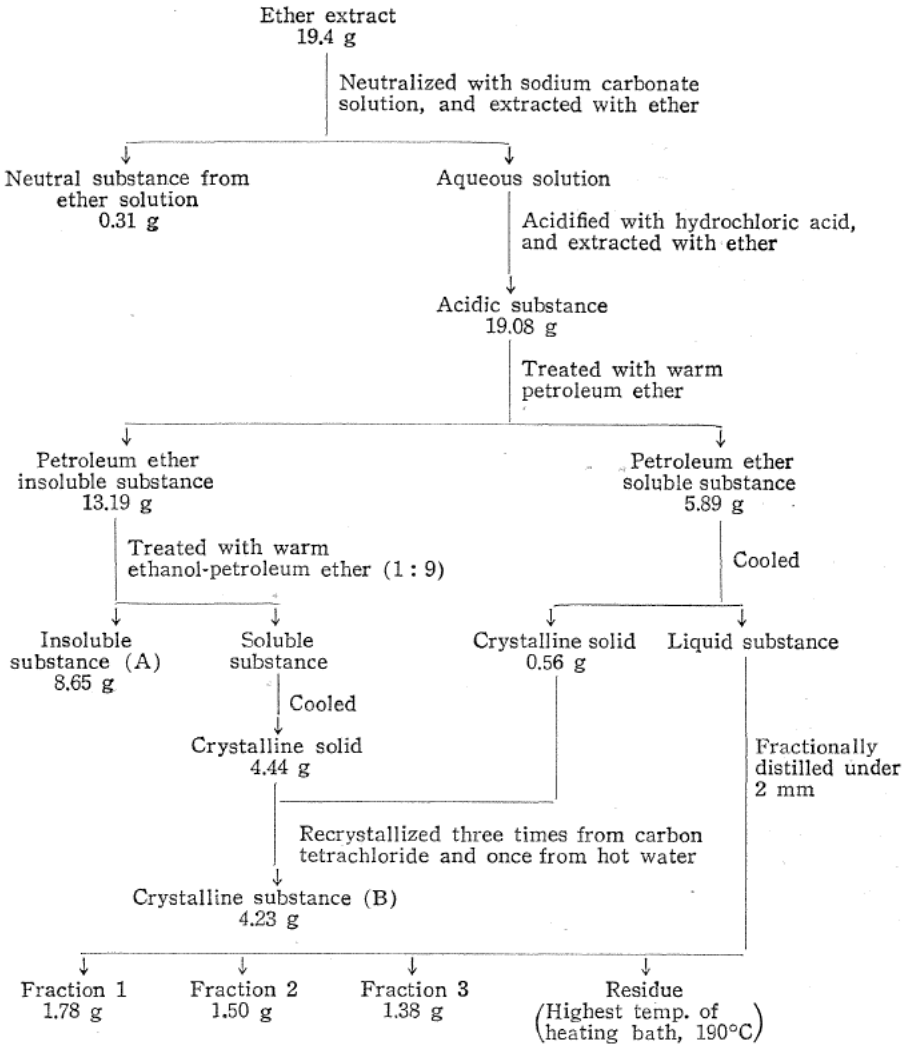
4. Fractionation of debrominated product. The debrominated product (methyl ester) was saponified, and the soap solution was acidified with dilute hydrochloric acid and then extracted with ether. The ether extract (19.4 g) was separated into several fractions according to the following scheme on the next page:

Insoluble substance A has N.V. 148.2, S.V. 248.2, Ester V. 100.0, I.V. 14.3, Acetyl V. 142.7, and Mol. Wt. (Rast) 542. This substance is composed chiefly of dimeric polymer as is seen from its molecular weight. High ester value and acetyl value indicate the presence of a lactone-like compound and free hydroxyl group. This substance is thought to be formed by a further rearrangement of the primary oxidation product formed at the early stage of oxidation.

Crystalline substance B has M.P. 105–106°C and N.V. 594.9, and is identified as azelaic acid (N.V., calculated, 596.2).

Fraction 1 has n_D^{15} 1.4207, N.V. 462.2 and I.V. 0.1, and consists chiefly of caproic acid (N.V., calculated, 483.0).

Fractions 2 and 3 have d_4^{15} 0.9161 and 0.9163, n_D^{15} 1.4469 and 1.4458, N.V. 362.1 and 358.2, and I.V. 72.8 and 76.9, respectively. Their neutralization values agree with the calculated value for nonenoic acid, 359.1. Also their specific refractivities, 0.2916 and 0.2909, agree with the calculated value for nonenoic acid, 0.2914. Both fractions consist presumably of nonenoic acid. Their iodine values are, however, considerably lower than the calculated value for nonenoic acid, 162.5, but this is explicable if the ethylenic linkage of the nonenoic acid is located near the carboxyl group, as is the case with β-nonenoic acid which is expected to be formed by the oxidative scission



at 9-ethylenic linkage of methyl linoleate. For such nonenoic acid, the Wijs method is known to give a considerably low iodine value.

Taking into consideration that cyclic peroxide as well as hydroperoxide is formed at the early stage of oxidation of methyl linoleate, and also that the conjugation of ethylenic linkage occurs, it is expected to yield monobasic and dibasic acids listed in Table 10, by the bromination of oxidation product followed by the oxidative scission of brominated product with potassium permanganate, the debromination of scission product and the saponification of debrominated product, as performed in the present experiments.

Since scission products in these experiments contain nonenoic acid (C_9, F_1) and azelaic acid in large proportions, the reaction (1) seems to occur predominantly. Although scission products to be formed from hydroperoxides are not known with

TABLE 10. Monobasic and Dibasic Acids from the Oxidation Product

Position of ethylenic linkages	Peroxide	Scission product	
		Monobasic acid	Dibasic acid
(1) 9, 12	9, 10-cyclic	$C_9(F_1)$	C_9
(2) 9, 12	12, 13-cyclic	C_6	$C_{12}(F_1)$
(3) 9, 12	8-hydroperoxide	$C_{11}(F_2)$, etc.	C_7-C_8
(4) 9, 12	11-hydroperoxide	?	?
(5) 9, 12	14-hydroperoxide	C_4-C_5	$C_{14}(F_2)$, etc.
(6) 9, 11	13-hydroperoxide	C_5-C_6	?
(7) 10, 12	9-hydroperoxide	?	C_8-C_9

certainty, they yield neither $C_9(F_1)$-monobasic acid nor C_9-dibasic acid in any substantial proportion. Since caproic acid (C_6) was formed in these experiments, the reaction (2) also occurs to a certain extent, but $C_{12}(F_1)$ dibasic acid which should be formed corresponding to caproic acid could not be identified in these experiments. This is explained if an assumption is permitted that when the reaction (2) occurs, 9-ethylenic linkage enters into intermolecular reaction so that monomeric $C_{12}(F_1)$ dibasic acid is not formed.

Anyhow, the above results indicate that among the primary oxidation products there are preponderantly those one in which 9-ethylenic linkage underwent oxidation while 12-ethylenic linkage remained intact.

IV. The Course of Oxidation of Methyl Linolenate[21]

The course of oxidation of methyl linolenate was studied in a similar way as described in the preceding experiments with methyl linoleate.

1. Preparation of methyl linolenate. The mixed fatty acids of linseed oil were brominated in ether, and the insoluble hexabromide melting at 182°C was separated in a yield of 42%. The hexabromide was debrominated with zinc and sulfuric acid in methanol, and the resulting methyl ester was distilled. The fraction boiling mainly at 184°C under about 4 mm was caught as methyl linolenate. It had S.V. 192.4 and I.V.* 261.0. Calculated for methyl linolenate: S.V. 191.8 and 260.4.

2. Oxidation of methyl linolenate. To a solution of 34 g of methyl linolenate in 200 cc of glacial acetic acid was added 0.37 g of cobalt resinate, and the solution was exposed to an atmosphere of oxygen using a shaking apparatus in the absence of light at room temperature of 15.8–20°C. After the elapse of 4.5 hours of induction period, oxygen absorption began and about 2,700 cc of oxygen was absorbed in the course of an over-all 18.5 hours. This oxygen absorption is close to the calculated value which corresponds to the absorption of one mole of oxygen per mole methyl linolenate. After oxygen absorption, the product was extracted with a large quantity of ether, the ether solution was freed from acetic acid by washing with water, and on distilling off the ether, there was obtained 37.59 g of an oily liquid having a penetrating odour and the following characteristics: A.V. 11.7, S.V. 318.1, Hydroxyl V. 122.6, Diene V. 6.26, Carbonyl V. 32.3, I.V. 112.7, Peroxide V.** 487.5.

* While the iodine values recorded in I, II, III, and VI were determined by the Wijs method, those recorded in IV and V were determined by the pyridine sulfate dibromide method.

** The figures for peroxide value were obtainable immediately after addition of the reagent.

3. Bromination of oxidation product, oxidative scission of brominated product with potassium permanganate, and debromination of scission product. The oxidation product (33.79 g) was brominated in ether under cooling, and there was obtained 8.90 g of insoluble bromide with M.P. 157°C and Br-content 61.95% (calculated for methyl linolenate, 62.12%), which was hexabromide formed from the unchanged methyl linolenate. The bromide soluble in ether had Br-content 42.87%. To a solution of 53.30 g of this bromide in 600 cc of acetone was added 180 g of potassium permanganate in small portions in the course of 3.5 hours, and the mixture was refluxed for 10 hours. After distilling off the acetone, the residue was treated with sulfurous acid in order to remove the insoluble oxides of manganese and the excess of potassium permanganate. The scission product was extracted with ether. As a part of the scission product remained in aqueous layer, it was evaporated to dryness after neutralization with potassium hydroxide, and the dried residue was extracted with ethanol. From the ethanol solution ethanol was distilled off, the residue was acidified with hydrochloric acid and then thoroughly extracted with ether. The scission product obtained by this extraction was combined with the main portion obtained above. The combined scission product had Br-content 38.08% and I.V. 5.03. It was debrominated with zinc and sulfuric acid in methanol, and there was obtained an oily liquid having S.V. 411.5, I.V. 71.0 and Hydroxyl V. 233.2.

4. Fractionation of debrominated product and examination of each fraction. The debrominated product thus obtained was separated into several fractions according to the following scheme on the next page:

The unsaponifiable matter (A) seems not to be a primary oxidation product; it is possibly a product formed from the primary oxidation product by decarboxylation and other reactions. It is a semi-solid having I. V. 43.6, Hydroxyl V. 169.5 and Carbonyl V. 150.3, and is partly insoluble in 90% ethanol.

The neutral substance (B) is an oily liquid of lactone nature having S.V. 221.5. The substance insoluble in ethanol-petroleum ether (C) is a dark brown viscous oil having A.V. 194.9, S.V. 261.4, I.V. 64.7, Hydroxyl V. 256.1, and Mol. Wt. by Rast 659 and is considered to consist chiefly of dimeric oxidation product.

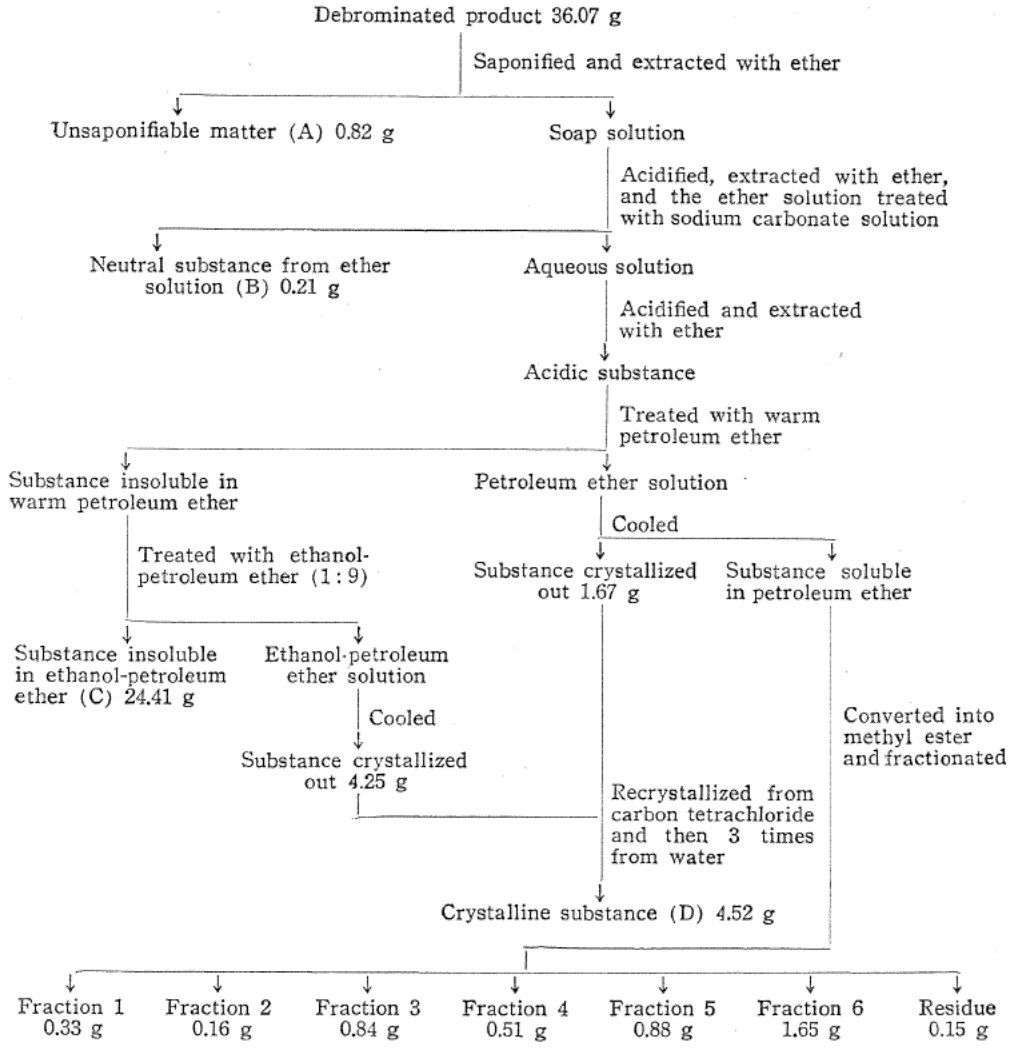
The crystalline substance (D) is azelaic acid having M.P. 105–106°C and N.V. 595.9 (calculated, 596.2).

Fraction 1 was collected as the first running by the distillation at ordinary pressure. It is a colorless liquid having a characteristic odour of methyl esters of lower fatty acids. It shows no iodine value, and its S.V., 600.9 seems to indicate that Fraction 1 is composed of methyl esters of propionic and butyric acids (saponification value calculated for methyl propionate and butyrate: 636.8 and 549.3).

Fractions 2 and 3, B.P. 80–89°C and 89–105°C under about 2 mm, respectively, were combined and redistilled. A fraction (a) boiling at 85–103°C under about 2 mm was separated. This was a colorless liquid and had the following characteristics:

	S.V.	I.V.	Hydrogen V.
Fraction (a)	466.7	25.2	164.2
Calculated for methyl pentenoate	491.5	222.4	176.6
Calculated for methyl hexenoate	437.7	198.1	157.3

Saponification value of the fraction (a) lies between the calculated values for methyl pentenoate and hexenoate, but iodine value of the fraction (a) is very small



compared with the calculated values. Thus the ethylenic linkage in the fraction (a) does not add bromine quantitatively. This may be explained by assuming the ethylenic linkage in the fraction (a) at the α- or β-position.

Fraction 4, boiling mainly at 130–138°C under about 2 mm, and Fraction 5, boiling at 138–144°C under about 2 mm, had the following characteristics:

	S.V.	I.V.	Hydrogen V.
Fraction 4	340.1	108.4 (168.0)	237.1
Fraction 5	326.8	104.4 (178.9)	244.4
Calculated for methyl nonadienoate	333.5	301.8	239.7

Iodine values in the parentheses were determined by the modified pyridine sulfate dibromide method.[22]

Although Fractions 4 and 5 are not composed exclusively of methyl esters of C_9-

acids, the two fractions correspond closely to methyl nonadienoate. Low iodine values of the two fractions compared with the calculated value may be ascribed to the presence of ethylenic linkage which does not enter into reaction in the iodine value determination. The absorption spectra of Fraction 4 showed extinction coefficients ($E^{1\%}_{1cm}$) 83.7 at 235 mμ and 76.5 at 263 mμ.* The corresponding values of Fraction 5 are 7.2 and 5.2, respectively. Hence the amount of conjugation in Fractions 4 and 5 is small. The oxidation product of the fatty acid from Fraction 4 by Hazura's method melted at 123–124.5°C and had N.V. 187.8 and S.V. 257.1 (calculated for tetrahydroxynonoic acid: 252.5). This fact suggests that a part of ethylenic linkages in Fraction 4 lies near the carboxyl group and the hydroxy derivative is liable to form lactone.

Fraction 6, boiling mainly at 180–192°C/3 mm, consists chiefly of regenerated methyl linolenate as is seen from its S.V. 195.8 and I.V. 265.2.

V. The Course of Oxidation of Methyl Clupanodonate

In a continuation of the preceding study, the course of oxidation of methyl clupanodonate was studied in a similar way.

1. Preparation of methyl clupanodonate. Sardine oil of S.V. 195.8 and I.V. 172.6 was subjected to the sodium salt acetone method,[23] and a concentrate of highly unsaturated acids with N.V. 179.7 and I.V. 320.8 was separated. This was esterified with methanol, the methyl ester was fractionated, and there was obtained a higher fraction boiling above 180°C/1 mm and having I.V. 331.6 in a yield of 55% of methyl ester. Further fractionation of the higher fraction gave a fraction boiling at 200–210°C/1 mm in a yield of 34% of the higher fraction. It had S.V. 164.3 and I.V. 370.5 (calculated for methyl clupanodonate $C_{23}H_{36}O_2$: S.V. 162.8, I.V. 368.4). Although this fraction may not be thought as pure methyl clupanodonate, it is certain that this fraction consists chiefly of methyl clupanodonate, *i.e.*, methyl ester of 4, 8, 12, 15, 19-docosapentaenoic acid. Curve I of Fig. 1 represents the ultraviolet absorption spectrum of this fraction in ethanol. It shows typical absorption intensities for conjugated triene having maximum absorption at 275 mμ and minimum absorptions at 269 and 281 mμ. The three wave lengths indicated are, however, longer by a few units than the corresponding wave lengths reported for conjugated triene of C_{18}-acids. It exhibits also a discernible absorption at 236 mμ, while oxidized methyl esters, as shown in Curves II and III, Fig. 1, exhibit a well defined maximum at 236 mμ for conjugated diene. This is also longer by a few units than the corresponding wave length reported for C_{18}-acids. Because of the lack of standard data on the ultraviolet absorption characteristics for conjugations of C_{22}-acids, conjugations in methyl clupanodonate can not be estimated satisfactorily by the spectrophotometric method. But if the standard extinction coefficients for conjugated diene and triene in this case be assumed to be the same with the corresponding values for C_{18}-acids, and also if the method of calculations proposed by Brice and Swain[24] be assumed to be applicable in this case, the conjugated diene and triene in methyl clupanodonate obtained above are found to be 4.1% and 2.2% respectively from the data shown in Curve I, Fig. 1; $E^{1\%}_{1cm}$ 39.38 at 236 mμ for conjugated diene; $E^{1\%}_{1cm}$ 62.59, 71.91 and 55.06

* The absorption at 263 mμ may presumably be ascribed to the diene conjugation at the α-position.

at 269, 275 and 281 mμ, respectively, for conjugated triene. Such amounts of conjugated diene and triene in methyl clupanodonate are thought to have been formed in the course of the procedures, such as saponification and distillation, for the preparation of methyl clupanodonate.

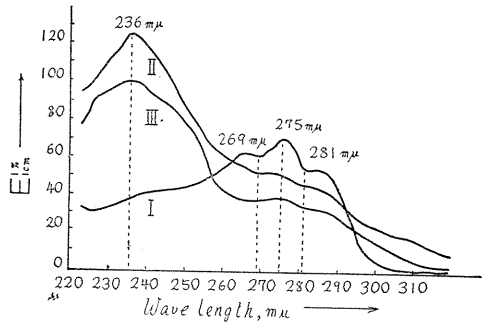


FIG. 1. Ultraviolet absorption spectra of methyl clupanodonate and oxidized esters.

2. Oxidation of methyl clupanodonate. Twenty-six g of methyl clupanodonate was dissolved in 200 cc of glacial acetic acid, 0.8 g of cobalt resinate was added, and the solution was exposed to an atmosphere of oxygen using a shaking apparatus in the absence of light at room temperature of 22–26°C. Fig. 2 represents the rate of

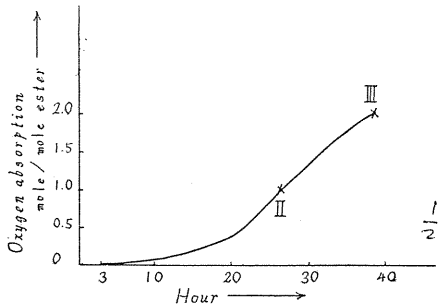


FIG. 2. Rate of oxygen absorption.

oxygen absorption. The oxygen absorption was one mole per mole methyl clupanodonate after 26 hours and 40 minutes, including an induction period of 3 hours, and two moles after 38 hours and 40 minutes. The properties of oxidized esters

TABLE 11. Properties of Oxidized Ester

	Methyl clupanodonate (I)	Oxidized ester, absorbed one mole of oxygen (II)	Oxidized ester, absorbed two moles of oxygen (III)
Appearance	Nearly colorless	Yellowish	Yellowish, viscous
d_4^{15}	0.9250	—	—
n_D^{15}	1.4927	1.4991	1.5009
Iodine value, apparent	—	252.8	202.2
Iodine value, true	370.5	280.9	236.7
Peroxide value	—	1114	1571
Acid value	2.9	23.5	48.9
Saponification value	164.3	188.4	293.6
Hydroxyl value	—	274.8	253.1
Conjugated diene (%)	4.1	13.3	10.7
Conjugated triene (%)	2.2	0.2	2.0

together with those of original ester are recorded in Table 11.

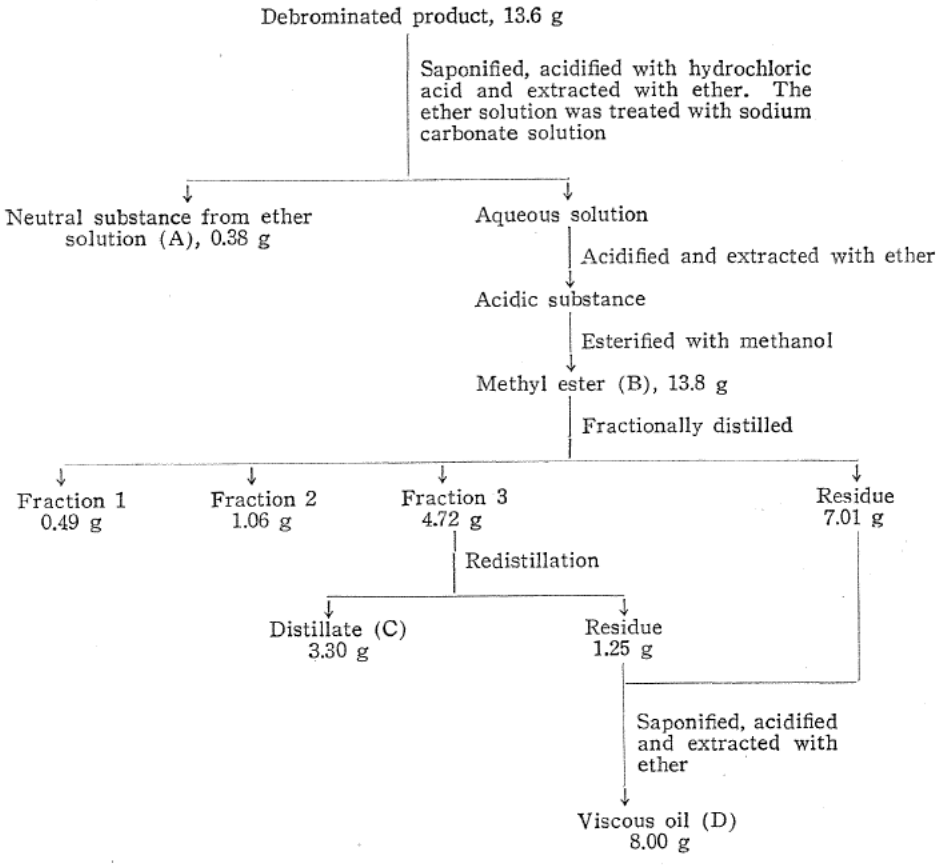
Apparent iodine value of oxidized ester is the value obtained in the usual way, while true iodine value is the value obtained after correction for the amount of iodine liberated by peroxide in oxidized ester.

As is seen from Table 11, iodine value of methyl clupanodonate falls with the progress of oxidation. If oxygen absorption is assumed to occur exclusively at ethylenic linkages in such a way that each ethylenic linkage is saturated with one mole of oxygen, the calculated iodine values for oxidized esters (II) $C_{23}H_{36}O_4$ and (III) $C_{23}H_{36}O_6$ will be 269.7 and 186.4, respectively. Compared with these calculated iodine values, the true iodine values of II and III, especially of III, are higher. Accordingly, the absorbed oxygen must have been consumed not only to saturate ethylenic linkages but also to other reactions among which the formation of hydroperoxide at α-methylenic carbon is conceivable. Peroxide value is still increasing in the region of oxygen absorption of one moles, while conjugated diene decreases from 13.3% to 10.7% in the same region. A similar fact was reported in the case of the oxidation of methyl linoleate and linolenate at 20°C by Gunstone and Hilditch[25] who observed that conjugated diene attains maximum before peroxide value becomes maximum. The changes of conjugated triene in the course of oxygen adsorption up to two moles are rather slight.

3. Bromination of oxidized methyl clupanodonate (II). Twenty-four g of oxidized methyl clupanodonate (II) was brominated in ether solution under cooling below 0°C, and the ether-insoluble and ether-soluble bromides were separated. The ether-insoluble bromide was then treated with benzene in order to separate it into the benzene-insoluble and benzene-soluble bromides. The benzene-insoluble bromide (27 g) was found to de decabromide derived from unchanged methyl clupanodonate. It had Br-content 70.41% (calculated for $C_{23}H_{36}O_2Br_{10}$, 69.88%). Hence, at least 30% of methyl clupanodonate must have remained intact in the course of oxidation.

4. Oxidative scission of brominated product. The ether-soluble bromide and the benzene-soluble bromide, 32 g in total, were dissolved in 400 cc of glacial acetic acid, and 170 g of potassium permanganate was added in small portions. The mixture was heated for 10 hours on a water bath. After the completion of reaction, acetic acid was removed under vacuum, the residue was added with 1 litre of water and treated with sulfurous acid in order to remove the insoluble oxides of manganese and the excess of potassium permanganate. The scission product was extracted from the aqueous solution using a large quantity of benzene. In order to recover the scission product remaining in the aqueous solution, it was neutralized with sodium carbonate, and concentrated to a volume of 100 cc. Solid matter deposited in the course of concentration was filtered at several intervals. The solution was then evaporated further to dryness. The residue was extracted with ethanol, the ethanol solution after concentration was added with sulfuric acid and refluxed, by which the acidic scission product dissolved in ethanol was converted into ethyl ester. The latter was extracted using ether. After removal of ether, there remained an oily liquid (0.52 g) having n_D^{15} 1.4218, S.V. 640.1 and I.V. 0, which was found to be diethyl succinate (S.V. calculated for diethyl succinate, 644.2).

5. Debromination of the scission product. The scission product extracted with benzene was a brownish viscous liquid; 30.5 g, Br-content 55.34%. This was debrominated with zinc and sulfuric acid in methanol, and the debrominated product was separated into several fractions as shown in the following scheme:



Figs. 3 and 4 show ultraviolet absorption curves of each fraction recorded above. Fig. 5 shows ultraviolet absorption curves of Fraction 2, Distillate C and Viscous oil D after alkali-isomerization at 180°C for 30 minutes using a 1.3 N solution of potassium hydroxide in ethylene glycol.

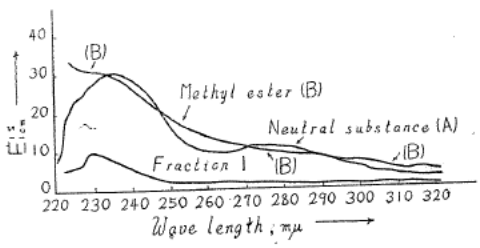


FIG. 3. Ultraviolet absorption spectra of scission products.

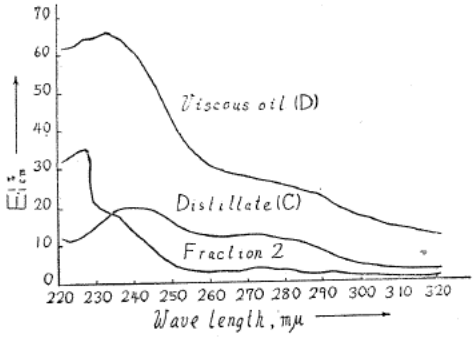


FIG 4. Ultraviolet absorption spectra of scission products.

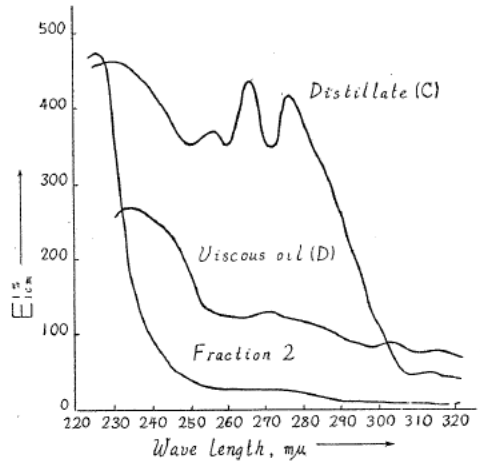


FIG. 5. Ultraviolet absorption spectra of scission products after alkali-isomerization.

6. Examination of scission product. Each fraction recorded above was examined below.

Neutral substance (A).—This was of jelly-like consistency and had S.V. 204.9, I. V. 139.5 and Mol. Wt. by Rast 548. It is believed from saponification value and molecular weight that this substance consisted of dimeric lactone. The molecular weight calculated on the basis of saponification value was 547.7 which shows a close agreement with the molecular weight determined by the Rast method. The I.V. 139.5 seems to indicate the presence of three ethylenic linkages in this dimeric lactone, since the calculated iodine value for a dimeric lactone having Mol. Wt. 547.7 and three ethylenic linkages is 139.0.

Fraction 1.—This fraction was a liquid having fruit aroma and had B.P. below 110°C/5 mm, d_4^{15} 0.9405, n_D^{15} 1.4321, S.V. 460.3 and I.V. 40.7. Absorption spectrum of this fraction exhibited only a very weak maximum, $E_{1\,cm}^{1\%}$ 9.94, at 228 mμ, from which diene conjugation in this fraction is estimated to be very small, say less than 1%. Although the molecular refraction of this fraction, 35.44, shows a good agreement with the calculated value for methyl hexenoate, 35.59, the saponification value of this fraction, 460.3, is higher than the calculated value for methyl hexenoate, 437.7. Higher saponification value of this fraction is presumably attributable not to the presence of methyl esters of monobasic acids lower than C_6-series, but to the presence of dimethyl succinate in this fraction. The S.V. 460.3 corresponds to a mixture of 93.16% of methyl hexenoate and 6.84% of dimethyl succinate or a mixture of 82.38% of methyl heptenoate and 17.62% of dimethyl succinate. Iodine value of Fraction 1 was very small compared with the calculated value for methyl hexenoate or heptenoate, but this is explicable if the ethylenic linkage of methyl ester is located at β- or γ-position.

From these results, it may presumably be concluded that Fraction 1 consists chiefly of methyl hexenoate or heptenoate possibly together with a lesser amount of dimethyl succinate and also some other minor components.

Fraction 2.—This fraction had B.P. 110–160°C/5 mm, d_4^{15} 1.0101, n_D^{15} 1.4611, S.V. 469.9 and I.V. 137.3. It should be noted that this fraction has a very high specific gravity and also that the saponification value of this fraction is a little higher than that of Fraction 1. These facts seem to indicate that this fraction contains methyl

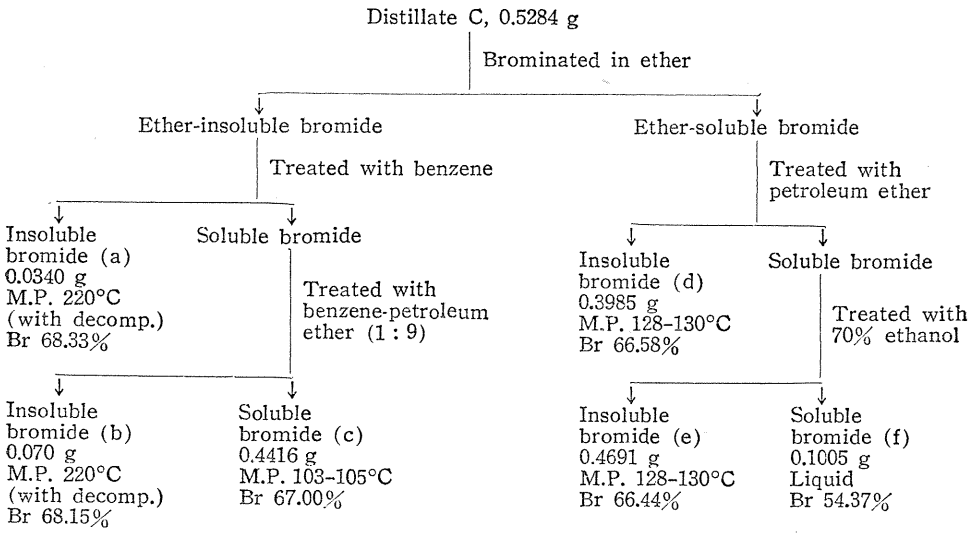
esters of monobasic acids and dimethyl succinate, although the identification of the latter could not be attained here. This fraction was hydrogenated, the product was saponified, and the free fatty acids were obtained in the usual way by using ether. The yield of fatty acids were only 60.5% of Fraction 2, which indicates that a considerable proportion of fatty acids was removed in the course of washing the ether solution with water. Fractional crystallization of the fatty acids from aqueous ethanol gave capric acid of M.P. 31–31.5°C and possibly also nonoic acid of M.P. 12–12.5°C. Assuming that Fraction 2 consists of the methyl esters of C_{10}- and C_9-monobasic (diunsaturated) acids and dimethyl succinate, and that capric and nonoic acids were recovered quantitatively from the hydrogenated product while succinic acid was lost entirely, the content of dimethyl succinate in Fraction 2 is found to be approximately 36%, calculating from the yield (60.5%) of the fatty acids of hydrogenated product. On the other hand, the S.V. 469.9 of Fraction 2 corresponds to a mixture of 65% of methyl decadienoate and 35% of dimethyl succinate or a mixture of 69% of methyl nonadienoate and 31% of dimethyl succinate. Thus the figures for the content of dimethyl succinate calculated on the basis of saponification value are in agreement with those calculated from the yield of the fatty acids of hydrogenated product. While Fraction 2 shows a weak maximum absorption, $E^{1\%}_{1\,cm}$ 34.61, at 227 mμ, it shows a strong maximum absorption, $E^{1\%}_{1\,cm}$ 470.2, at 227 mμ after alkali-isomerization, no appreciable absorption being observed in any other region. These spectral characteristics indicate that Fraction 2 contains a large proportion of non-conjugated diene with a very small proportion of conjugated diene. It may be concluded from these results that Fraction 2 consists substantially of methyl decadienoate and nonadienoate, possibly with 31–35% of dimethyl succinate. While the iodine value for a methyl ester mixture of the above composition is expected to be 181–208, the iodine value of Fraction 2 is relatively low. This may be attributed to the presence of unsaturated linkages which are located near the ester group.

Distillate C.—B.P. 162–168°C/5 mm, d_4^{15} 0.9348, n_D^{15} 1.4835, S.V. 232.2, I.V. 296.1. Fatty acids of the hydrogenated product of this fraction were fractionally crystallized from ethanol, and myristic acid of M.P. 53.5–54°C and stearic acid of M.P. 70°C were separated, the latter being small in quantity. Also the presence of pentadecanoic acid together with a minor amount of acids lower than myristic acid was indicated, though these could not be separated. As shown below, this fraction as a whole seems to consist chiefly of methyl tetradecatrienoate and pentadecatrienoate.

	Molecular refraction		Saponification value	Iodine value
Distillate C	72.27	76.56	232.2	296.1
Methyl tetradecatrienoate	71.60		237.7	322.2
Methyl pentadecatrienoate		76.22	224.1	304.2

Brominated product of this fraction was fractionated as shown below on the next page :

An inspection of Br-content of each bromide fraction indicates that the bromide fractions (a) and (b) are substantially octabromide of methyl octadecatetraenoate (calculated, Br 68.03%), while the bromides (c), (d), and (e) consist chiefly of hexabromide of methyl tetradecatrienoate (calculated, Br 66.98%) or methyl pentadecatrienoate (calculated, Br 65.69%). In the bromide (f), there should be contained some tetrabromides. Spectrophotometric examination of Distillate C indicates the presence of about 2% of diene conjugation, $E^{1\%}_{1\,cm}$ 17.22 at 230 mμ. Triene and tetraene



conjugations are absent or present in negligible amount. Absorption spectrum of the alkali-isomerized product exhibits $E^{1\%}_{1\,cm}$ 464.40, 345.11, 437.83, 343.99, 48.33, 48.89 and 37.96 at 230, 261, 266, 271, 310, 316 and 322 mμ, respectively, from which non-conjugated diene, triene, and tetraene in Distillate C are estimated at 2%, 88%, and 8%, respectively. Above spectrophotometric analyses were carried out by assuming the standard specific extinction coefficients in this case be the same with the corresponding values reported for C_{18}-acids and arachidonic acid.

From these results, Distillate C is believed to consist mainly (approximately 90 %) of non-conjugated triene (methyl tetradecatrienoate possibly with pentadecatrienoate) associated with small amounts of non-conjugated tetraene (possibly methyl octadecatetraenoate) and non-conjugated diene (possibly methyl decadienoate and nonadienoate found in Fraction 2).

Viscous oil D.—A.V. 166.8, S.V. 238.3, I.V. 134.1, Hydroxyl V. 10.7, Mol. Wt. by Rast 567.3. These data indicate that this fraction consists chiefly of dimeric acids and estolides. This fraction exhibits a maximum absorption, $E^{1\%}_{1\,cm}$ 66.22, at 234 mμ, indicating the presence of diene conjugation. The alkali-isomerized product shows a strong maximum, $E^{1\%}_{1\,cm}$ 265.00, at 234 mμ, and also a weak but typical absorption of tetraene conjugation in the region of 310–322 mμ. But spectrophotometric analyses for the conjugations in this case could not be carried out, since the standard specific extinction coefficients of each conjugation for a dimeric compound like this fraction should differ quite markedly from the corresponding values for C_{18}- or C_{20}-monomeric acids.

VI. Oleic Acid and Its Esters Autoxidized in the Course of Long Storage[26]

Among the unsaturated acids of fatty oil, oleic acid is relatively stable against oxidation with air or oxygen. It undergoes oxidation, however, at a high temperature and even at ordinary temperatures when exposed to air for a long period. On exposing oleic acid with water in a sealed tube filled with oxygen to sunlight, Ciamician and Silber[27] found that it developed a rancid odour and the water became strongly acidic.

These authors identified nonoic acid and its lower homologue, azelaic acid and its higher homologue, and dihydroxystearic acid of M.P. 133°C in the oxidation product. Skellon[28] found dihydroxystearic acids of M.P. 95°C and 132°C, 10-hydroxystearic acid of M.P. 84°C, and iso-oleic acid together with a small amount of formic, acetic, and oxalic acids and acetaldehyde in the oxidation product which was obtained by blowing oxygen into oleic acid with or without a small amount of blown whale oil as a catalyzer. Ellis[29] found oxido-elaidic acid of M.P. 55.5°C, oxido-oleic acid of M.P. 59.5°C, and azelaic, suberic, oxalic, nonoic, and caprylic acids in the oxidation product obtained by blowing oxygen into oleic acid in the presence of 0.2% of cobalt oleate. Patrick and Emerson[30] oxidized oleic acid and its ethyl ester in a autoclave under air in the presence of chromium oxide as a catalyzer, and also in acetic acid solution with oxygen in the presence of a catalyzer consisting of acetates of manganese, lead, and cobalt. Among the oxidation products, they found monobasic acids from caproic to palmitic acid and dibasic acids from suberic to undecanedicarboxylic acid. Besides the literature cited above, there are many studies on the oxidation product of oleic acid and its esters, but few studies appear to have been made on oleic acid and its esters which were autoxidized in the course of long storage at room temperature.

The present author had available to him samples of oleic acid, methyl oleate, and ethyl oleate which had undergone autoxidation in the course of long storage. The results of an examination of the oxidation products in these samples are given here.

1. Ethyl oleate. The fatty acids of Japanese camellia oil were treated with lead salt alcohol method, and the liquid fatty acids were separated and converted into the lithium salts, which were then recrystallized from 50% ethanol. The fatty acids liberated from the recrystallized lithium salts were esterified with ethanol, and the ethyl esters were purified by fractional distillation giving a fraction of ethyl oleate which showed S.V. 180.7 (calculated, 180.7) and I.V. (Wijs) 81.8 (calculated, 81.7) immediately after its preparation. This sample of ethyl oleate (100 g) in a 300-cc flask stoppered loosely with a cork was stored in a case with glass door in this laboratory. During the storage, the highest room temperature was about 35°C, and the glass door was perhaps never exposed direct to the sun. After storage for 6 years, the sample became a little more viscous and its iodine value decreased to 62.3. Eighty g of this sample after storage was subjected to fractional distillation, by which there were obtained: the fraction (1), below 200°C/*ca.* 5 mm, 35 g; the fraction (2), 200–204°C/*ca.* 5 mm, 20 g; the residue, 20 g. The fraction (1) and the residue were examined further, while the fraction (2) appeared to consist substantially of unchanged ethyl oleate.

The fraction (1) had A.V. 41.8, S.V. 237.6, I.V. 57.8, Acetyl V. 102.1, and Peroxide V. 621.2. Thirty-two g of this fraction was fractionated further as shown in Table 12.

TABLE 12. Fractionation of the Fraction (1)

Fraction	B.P. (°C/10 mm)	Yield (g)	A.V.	S.V.	Fatty acids freed from unsaponifiable matter	
					N.V.	S.V.
1_a	90-120	2	103.8	227.6	356.5	357.3
1_b	120-160	6	194.0	318.0	318.6	320.0
1_c	160-190	4	141.3	280.4	280.3	281.8
1_d	190-210	12	73.5	239.8	238.8	240.2
Residue		7				

The acid values of each fraction were greater than the acid value of the ester before distillation. This was caused by partial decomposition of the oxidized products. Little or no unsaponifiable matter was contained in the above fractions except the fraction 1_a which had a considerable amount of unsaponifiable matter. Peroxide value decreased materially by distillation and disappeared completely after saponification of the fractions. The fatty acids from the fractions 1_c and 1_d deposited solid at room temperature. The amides were prepared from the fatty acids of each fraction and fractionally crystallized with the results shown in Table 13.

TABLE 13. The Amides from Each Fraction

Fraction	M.P. of amide
1_a	98 - 98.5°C
1_b	98 - 98.5°C and 102 -102.5°C
1_c	102-102.5°C and 104.5-105°C
1_d	104.5-105°C and 77.5- 78°C

The amide of M.P. 98–98.5°C was identified as nonoic acid amide by the mixed melting point test. The amides of M.P. 102–102.5°C and M.P. 104.5–105°C, it will be noted, are the same with lauric acid amide and myristic acid amide, respectively, in their melting point. The above two amides contain 7.10% and 6.00% of nitrogen, respectively, which agree with the calculated values, 7.02% and 6.16%, for lauric acid amide and myristic acid amide.

The residue (B.P. above 204°C/*ca.* 5 mm) had A.V. 22.0, S.V. 228.5, I.V. 44.3, Acetyl V. 121.0, Peroxide V. 94.4 and Mol. Wt. (Rast) 400. When the fatty acids liberated from 20 g of the residue were extracted with cold ether, there remained 1 g of insoluble solid, which after recrystallization from 80% ethanol had M. P. 132°C, N.V. 177.6, S.V. 177.5, and Acetyl V. 278.8 and was identified as dihydroxystearic acid (calculated, N.V. 177.2, Acetyl V. 280.3). The fatty acids soluble in cold ether were dissolved in ethanol and fractionally precipitated as lithium salts from the solution. The fatty acid (6 g) from the first precipitate was a viscous liquid having N.V. 200.1, S.V. 202.3, I.V. 15.6, Acetyl V. 17.3 and Mol. Wt. 549. This is considered to be a dimeric oxidation product. Dihydroxystearic acid was found also in the fatty acid (2.6 g, Acetyl V. 204.6) from the second precipitate and the fatty acid (2 g, Acetyl V. 202.3) from the third precipitate. The fatty acid contained in the final filtrate from the fractional precipitation of lithium salt was liquid, but on cooling the aqueous solution obtained by extracting with hot water, it deposited a small amount of solid substance which, though not closely examined, was deemed to be azelaic acid from its N.V. 586.7 (calculated, 595.1).

2. *Methyl oleate.* Methyl oleate prepared from Japanese camellia oil similarly as ethyl oleate had S.V. 189.3 (calculated, 189.2) and I.V. 86.2 (calculated, 85.6) immediately after its preparation. For the storage of this sample, a 500-cc bottle filled with it was corked tightly and placed in the same case which was used for the storage of ethyl oleate. The sample was taken out at several intervals in the course of 5 years for the purpose of other experiments so that there remained 70 g of sample after 5 years. When the sample was tested after 5 years, no significant changes in its characteristics were observed, but after a further 6 years' storage it showed S. V. 215.2 and I.V. 72.6. The sample after 11 years' storge (68 g) was fractionally distilled and there were obtained: the fraction (1), boiling below 198°C/*ca.* 10 mm,

23 g; the fraction (2) boiling at 198–204°C/*ca.* 10 mm, 29 g; the residue, 15 g.

The fraction (1) had A.V. 32.0, S.V. 235.1, I.V. 59.7, Acetyl V. 77.0, and Peroxide V. 800.1. Twenty g of this fraction was fractionated further with the results given in Table 14.

TABLE 14. Fractionation of the Fraction (1)

Fraction	B.P.	Yield (g)	A.V.	S.V.	Fatty aeids freed from unsaponifiable matter	
					N.V.	S.V.
1_a	–140°C/30 mm	2.1	122.2	302.4	332.1	334.2
1_b	140–165°C/30–25 mm	2.5	130.8	257.8	266.2	267.5
1_c	165–200°C/25–15 mm	3.8	43.5	229.0	229.6	230.5
1_d	200–210°C/15 mm	5.0	14.9	200.6	200.1	200.3
Residue		6.0				

As was noted in the case of ethyl oleate, the oxidation product of methyl oleate seems to have been decomposed during the distillation. Amides were prepared from the fatty acids freed from unsaponifiable matter of each fraction, and fractional crystallization of amides yielded nonoic and lauric acid amides from the fraction 1_a, lauric and myristic acid amides from the fraction 1_b, myristic acid amide from the fraction 1_c and the amide of unchanged oleic acid from the fraction 1_d.

The residue of the first distillation (B.P. above 204°C/*ca.* 10 mm) had A.V. 19.5, S.V. 209.5, I.V. 61.2, Acetyl V. 107.1, and Peroxide V. 166.8. On treating with petroleum ether, the fatty acids liberated from the residue were separated into three fractions: (1) most difficultly soluble, (2) difficultly soluble, and (3) soluble in petroleum ether. Recrystallization of the fraction (1) from 80% ethanol yielded dihydroxystearic acid of M.P. 132°C and N.V. 177.8. Since the fraction (1) had acetyl V. 168.2 and Mol. Wt. 470, it seemed to contain a dimeric polymer besides dihydroxystearic acid. The fraction (2) had Acetyl V. 47.8 and Mol. Wt. 292. On extracting with hot water and cooling the aqueous solution, a small amount of crystalline substance having N.V. 568.3 was separated, which was considered to consist chiefly of azelaic acid. The fraction (3) had Acetyl V. 158.4, Mol. Wt. 283, and Carbonyl V. 24.4. This fraction seemed to contain monohydroxystearic and monohydroxy-ketostearic acids together with dihydroxystearic acid.

3. *Oleic acid.* The sample of oleic acid was prepared by saponification of methyl oleate above described and had N.V. 200.1 and I.V. 88.9 immediately after its preparation. This sample (50 g) was placed in a 500-cc wide-mouth bottle loosely stoppered with a cork and stored for about 6 years in the same place as the sample of methyl oleate. After the storage, it showed N.V. 150.5, S.V. 219.0, I.V. 66.2, Acetyl V. 50.5, and Peroxide V. 46.2. Since the peroxide value in the case of ethyl and methyl oleates decreased or disappeared in the course of the procedures of distillation and saponification, the sample of oleic acid was fractionated in the following way which does not involve such procedures. It was dissolved in ethanol and fractionally precipitated as lithium salt by adding aqueous solution of lithium hydroxide. But the fatty acids liberated from each precipitate by acidification with hydrochloric acid showed lower peroxide values than the sample before these treatments, from which it is seen that peroxide was partly decomposed by lithium hydroxide. In the next place, fatty acids were fractionally precipitated as lead salts on adding a solution of lead acetate in 90% ethanol, in the place of lithium hydroxide solution, to the

sample dissolved in 90% ethanol. Acidification of each precipitate of lead salts with hydrochloric acid gave the fatty acids shown below.

Fatty acids from	1st ppt.	2nd ppt.	3rd ppt.	4th ppt.	Filtrate
Peroxide V	88.1	101.2	60.3	22.3	—

Fatty acids from lst ppt. yielded dihydroxystearic acid having M.P. 131–131.5°C, N.V. 177.8, and Acetyl V. 279.6 after recrystallization from 80% ethanol. Fatty acids from 2nd ppt. was subjected to repeated fractional precipitations of lead salts, and there was obtained from the filtrate a fatty acid fraction having a higher Peroxide V. 122.5, which after repeated treatment yielded a fatty acid fraction having a higher Peroxide V. 131.8 from the filtrate. Thus the repeated treatments yielded a fraction of more and more higher peroxide value. But the separation of a concentrate of peroxide could not be attained in these experiments.

VII. Summary

1. Each sample of oleic acid, elaidic acid, and 12- and 15-octadecenoic acids, the last-named two being chiefly of *cis*-form, was dissolved in acetic acid, to which was added cobalt resinate as a catalyzer. The solution was shaken in an atmosphere of oxygen at room temperature of about 30°C, the rates of oxygen absorption were measured and it was found that the rates of oxidation of the four acids showed no significant difference. The oxidation of the four acids with perbenzoic acid in chloroform proceeded, however, in different manners. The rates of oxidation of the oleic and elaidic acids were distinctly greater than those of the 12- and 15-octadecenoic acids. The rates of oxidation of the oleic and elaidic acids showed no significant difference, though the former may be but slightly greater. Likewise, the rates of oxidation of 12- and 15-octadecenoic acids showed no significant difference, though the former may be but slightly greater.

2. Similar experiments were carried out with oleic, linoleic, and linolenic acids. The average rate of oxygen absorption per one ethylenic linkage was found to be greatest for linolenic acid. Linoleic acid came next to linolenic acid, while oleic acid showed a markedly smaller rate of oxygen absorption. Oxidation of the three acids with perbenzoic acid in chloroform proceeded, however, in different manners. Comparing the average rate of oxidation per one ethylenic linkage, oleic acid showed a remarkably greater value than linoleic and linolenic acids.

3. Each sample of methyl linoleate, linolenate, and clupanodonate was oxidized to the extent that one mole of methyl ester absorbed approximately one mole of oxygen. The oxidized methyl ester was subjected to bromination, by which the ethylenic linkage, which escaped oxidation and was still capable of reacting with bromine, was saturated with bromine and protected against the oxidative scission in subsequent procedure. The brominated product was then subjected to oxidative scission using potassium permanganate, the scission product was debrominated, the debrominated product was separated into several fractions, and each fraction was examined

Methyl linoleate yielded relatively large amounts of azelaic and nonoic acids among monomeric acids, while there was no indication of the presence of unsaturated dibasic acid. Accordingly, it is concluded that in the monomeric primary oxidation products of methyl linoleate, those preponderated in which 9-ethylenic linkage was attacked by oxygen while 12-ethylenic linkage remained intact.

From methyl linolenate, there was obtained azelaic acid in a relatively large amount. Also the presence of saturated monobasic acids of C_3–C_4 series, monoethylenic monobasic acids of C_5–C_6 series and diethylenic monobasic acid corresponding nearly to C_9-series was indicated. There was no indication of the presence of unsaturated dibasic acid. These results indicate that in the monomeric oxidation products of methyl linolenate, as in the case of methyl linoleate, those preponderated in which 9-ethylenic linkage was attacked by oxygen while other ethylenic linkages remained partly or entirely intact.

Among the monobasic acids from methyl clupanodonate, the presence of monounsaturated acids of C_6–C_7, diunsaturated acids of C_9–C_{10}, triunsaturated acids of C_{14}–C_{15}, and tetraunsaturated acids of C_{18} was indicated. Succinic acid was identified, but no evidence was indicated for the presence of unsaturated dibasic acids. These results show that in the monomeric oxidation products of methyl clupanodonate, as in the case of methyl linoleate and linolenate, there are preponderantly those in which the ethylenic linkages located near the carboxyl group of clupanodonic acid were attacked by oxygen while the ethylenic linkages remote from the carboxyl group remained partly or entirely intact.

4. Nonoic, lauric, myristic, and azelaic acids, dihydroxystearic acid of M.P. 132°C, and dimeric oxidation product were separated from a sample of ethyl oleate which had undergone autoxidation in the course of long storage. In a sample of similarly autoxized methyl oleate, the presence of monohydroxystearic acid and monohydroxyketostearic acid besides the above mentioned compounds was indicated. However, these compounds might not have been originally present in the autoxidized oleates, since the autoxidized oleates were subjected to several procedures such as distillation and saponification for the separation and identification of these compounds. Peroxide values of the autoxidized oleates decreased markedly during distillation, and disappeared completely during saponification. Also a sample of similarly autoxidized oleic acid contained dihydroxystearic acid. In an attempt to obtain a concentrate of peroxides from this sample, it was fractionally precipitated by means of lead acetate from its ethanol solution, but the fractionation was not effective, thus giving a fraction of Peroxide V. 131.8 at most from the original sample of Peroxide V. 46.2.

References

1) Y. Toyama and T. Yamamoto: *J. Chem. Soc. Japan, Ind. Chem. Sect.* **55**, 164 (1952).
2) Geo. E. Holm, Geo. R. Greenbank, and E. F. Deysher: *Ind. Eng. Chem.* **19**, 156 (1929).
3) R. Kuhn and K. Meyer: *Z. physiol. Chem.* **185**, 193 (1929).
4) K. Täufel and E. Spiegelberg: *Chemische Umschau Fette, Oele, Wachse* **37**, 281 (1930); K. Täufel and A. Seuss: *ibid.* **41**, 107, 137 (1934).
5) S. Miyoshi and E. Ibuki: *J. Chem. Soc. Japan* **63**, 1151 (1942).
6) Ad. Grün and W. Czerny: *Ber.* **59**, 54 (1926).
7) Y. Toyama and T. Yamamoto: *J. Chem. Soc. Japan, Pure Chem. Sect.* **72**, 619 (1951).
8) C. R. Noller and R. A. Bannerot: *J. Am. Chem. Soc.* **56**, 1563 (1934).
9) Y. Toyama and T. Yamamoto: *J. Chem. Soc. Japan, Ind. Chem. Sect.* **55**, 176 (1952).
10) *l. c.*, 3).
11) *l. c.*, 4).
12) A. J. Stirton, J. Turner, and R. W. Riemenschneider: *Oil and Soap* **22**, 81 (1945).
13) T. P. Hilditch and J. J. Sleightholme: *J. Soc. Chem. Ind.* **51**, 39 T (1932).
14) Y. Toyama and I. Matsumoto: *J. Chem. Soc. Japan, Ind. Chem. Sect.* **54**, 383 (1951).
15) E. H. Farmer *et al.*: *Trans. Faraday Soc.* **38**, 340 (1942); *J. Chem. Soc.* **1943**, 119.

16) E. H. Farmer and D. A. Sutton: *J. Chem. Soc.* **1943**, 119; C. E. Swift, F. G. Dollear and R. T. O'conner: *Oil and Soap* **23**, 355 (1946).
17) D. Atherton and T. P. Hilditch: *J. Chem. Soc.* **1944**, 105.
18) W. O. Lundberg and J. R. Chipault: *J. Am. Chem. Soc.* **64**, 833 (1947).
19) W. Treubs: *Ber.* **75**, 1164 (1942).
20) S. Bergström: *Arkiv Kemi. Mineral. Geol.* **21** A, No. 14, 1 (1945); *Nature* **156**, 717 (1945); *C. A.* **40**, 1450, 6410 (1946).
21) Y. Toyama and I. Matsumoto: *Fette und Seifen* **53**, 523 (1951).
22) G. H. Benham and L. Klee: *J. Am. Oil Chemists' Soc.* **27**, 127 (1950).
23) Y. Toyama and T. Tsuchiya: *J. Soc. Chem. Ind. Japan* **28**, 262 (1925).
24) B. A. Brice and M. L. Swain: *J. Opt. Soc. Am.* **35**, 532 (1945).
25) F. D. Gunstone and T. P. Hilditch: *J. Chem. Soc.* **1945**, 863.
26) Y. Toyama and T. Yamamoto: *J. Chem. Soc. Japan, Ind. Chem. Sect.* **55**, 235 (1952).
27) G. Ciamician and P. Silber: *Ber.* **47**, 640 (1914).
28) J. H. Skellon: *J. Soc. Chem. Ind.* **50**, 382 (1931).
29) G. W. Ellis: *Biochem. J.* **30**, 753 (1936).
30) T. M. Patrick, Jr., and W. S. Emerson: *Ind. Eng. Chem.* **41**, 636 (1949).