Preparation of the Sterols: The sterol acetates are saponified with the aid of a little (about 3 ml.) half normal alcoholic potassium hydroxide. After dilution with water the sterols are extracted with ethyl ether. The solution is washed once or twice with a little water and then the ether is evaporated. The sterols obtained are dissolved in 1 or 2 ml. of 96% alcohol.

The sterols are crystallized on a microscope slide. The form of the crystals, as well as their extinction of polarized light, is observed under the microscope. Crystals of phytosterol are oblong and longer than wide and show parallel extinction while those of cholesterol are wider and show inclined extinction.

SUPPLEMENT II_{5A}

Characterization of Sterols

Reagents:

1. Alcoholic potassium hydroxide, 200 g. KOH per liter of 70% alcohol.
2. Hydrochloric acid, 25% solution.
3. Digitonin solution, 1 g. in 100 ml. 96% alcohol.
4. Chloroform.
5. Acetic anhydride.
6. Alcohol.
7. Ethyl ether.
8. Alcoholic potassium hydroxide, approximately N/2.

Preparation of Fatty Acids: One hundred g. of fat are saponified with 200 ml. of the alcoholic potassium hydroxide solution in a flask under reflux. When the saponification is complete, dilute the soap solution with 300 ml. of hot water. Add an excess of hydrochloric acid. Heat until the fatty acids form a clear film on the surface. Pour the mixture on a previously moistened filter paper. When the aqueous liquid has filtered, pierce the filter. The fatty acids are received in a beaker. Add 30 ml. of chloroform and filter the solution through a large dry folded paper.

Preparation of the Digitonides: The fatty acids are heated in a beaker to 70°C. and, while stirring, add at once 50 ml. of the solution of digitonin. The mixture is reheated to 70°C. and stirred at that temperature for 15-20 minutes with a glass rod. Add another 30 ml. of chloroform and filter the mixture on a small filter with suction. The crystals of digitonide are washed three times with 20-ml. portions of hot chloroform and five times with 15-ml. portions of ethyl ether. Afterwards they are dried on a watch glass at 100°C. for 10 minutes.

Purification of the Crystals: Dissolve the crystals in 100 ml. of absolute alcohol, heat, and add 10 ml. of water. Allow the mixture to stand overnight and then add 15 ml. of water. Let stand for an hour and filter on a small suction filter. The crystals are dried at 100°C. for 10 minutes.

Preparation of Sterol Acetates: Digest the crystals for 10 minutes with 7 ml. of acetic anhydride in a flask under reflux until the solution becomes clear. Add 28 ml. of 50% alcohol. Place the flask in cold water for an hour. Filter on a small suction filter. Wash the crystals with 50% alcohol.

Dry the crystals on a watch glass at 100°C. for 10 minutes. Recrystallize several times from absolute alcohol. A porous porcelain plate will serve to absorb the alcohol. After the second crystallization determine the melting point.

Preparation of the Sterols: The sterol acetates are saponified with 2-3 ml. of N/2 alcoholic potassium hydroxide solution. The sterols so produced are collected on a porous porcelain plate. They are recrystallized from absolute alcohol and microscopically examined under both normal and polarized light.

Observations on the Mechanism of the Autoxidation of Methyl Linoleate[1,2]

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Introduction

ALTHOUGH a number of people have studied extensively the autoxidation of methyl linoleate and other simple esters of linoleic acid, the structures of the peroxides that are formed during the initial stages of the autoxidation have never been completely established. It is the purpose of this paper to present a few preliminary observations obtained in a study of the autoxidation of methyl linoleate that was undertaken to gain further evidence concerning the structures of the initial peroxides.

Before presenting the data, however, it is advisable to review briefly some of the findings that have been reported by other investigators. It appears to have been well established by Farmer and others (1,2,3,4) that the autoxidation of olefins occurs by a free radical mechanism which, in some cases at least, results ultimately in the formation of hydroperoxides in which the peroxidic group is attached to a carbon atom adjacent to an unsaturated center. Kass *et al.* (5), Mitchell *et al.* (6), Farmer *et al.* (7), and others (8,9) have also observed by spectrophotometric methods that, in polyolefins whose double bonds are separated by methylenic groups, treatment with alkali at higher temperatures or autoxidation at ordinary temperatures apparently causes some double bonds to shift, thereby producing some conjugation of the double bond systems.

Bolland and Koch (10), Bergström (8), and also several people in this country have devoted considerable attention to the conjugation that develops during the autoxidation of linoleates. Bolland and Koch, in particular, on the basis of studies of the autoxidation of ethyl linoleate, postulated the formation of a

[1] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 231 in the series of papers approved for publication. The views and conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army. (Hormel Institute Publication No. 35.)

[2] Presented at the meeting of the American Oil Chemists' Society, New York, November 15-17, 1948.

resonating linoleate free radical by the removal of a hydrogen from the methylenic carbon between the two double bonds. It was pictured that, in addition to its original unconjugated form, this radical could assume either of two conjugated dienoic forms which could be stabilized by the formation of hydroperoxide groups at the 9 or 13 carbon positions.

One important observation made by these workers, and also by Bergström (8), was that the ultraviolet spectral absorption of linoleate peroxides at 2315 Å was appreciably lower than would be anticipated if conjugated diene groups and peroxide groups were formed in a 1:1 molar ratio. On the basis of the then available extinction coefficients of conjugated dienoic systems Bolland and Koch estimated that their linoleate peroxides were between 70 and 85% conjugated. They drew no definite conclusions regarding the chemical structure of the unconjugated peroxides except that they were considered to be hydroperoxides. Their results left the door open, however, to the attractive but unsubstantiated conclusion that the low absorption was due to the presence of some 11-hydroxyperoxide containing no conjugated unsaturation.

Bergström, on the other hand, obtained chemical data which suggested that little or no 11-hydroperoxide or other unconjugated monohydroperoxide was formed. After hydrogenating autoxidized methyl linoleate and chromatographing the products, he was able to isolate 9- and 13-monohydroxystearates but no 11-monohydroxystearate. Lead tetra-acetate titrations also revealed about 0.2 mols. of alpha glycolic groups in the hydrogenated products for each mol. of peroxide that had been formed. In another experiment, when the peroxides were first fractionated on an adsorption column and then hydrogenated, he found that some of the fractions contained hydroxy derivatives with two or more hydroxyl groups per molecule.

Bergström pointed out, however, that chemical changes might have taken place either during the hydrogenation or the adsorption processes that could account for the failure to find the 11-monohydroxystearate. His results, therefore, left it an open question whether any 11-monohydroperoxide or other unconjugated hydroperoxide is formed to any appreciable extent during the autoxidation of methyl linoleate.

Bolland (11) and Bolland and Gee (12) made quite extensive studies of the kinetics of the autoxidation of ethyl linoleate. After making calculations based on the bond energies and resonance energies that are involved, they came to the conclusion that either of the conjugated diene radicals with a free linkage at the 9 or 13 position is more stable than the unconjugated radical with a free linkage at the 11 position, and on this basis it would appear that there would be a tendency against the formation of any appreciable amount of the unconjugated 11-hydroperoxide.

Lundberg and Chipault (13) studied the oxidation of methyl linoleate at various temperatures between 40 and 100°C. and in the presence of positive and negative catalysts. They found that when samples of linoleate from the same batch were used, in all cases the ratio of the spectral absorption at 2325 Å to the peroxide content was constant during the early stages of the reaction, irrespective of the temperature and other conditions. Bergström and Holman (14) found a molecular extinction of 31,400 for the conjugated linoleate peroxides formed in the lipoxidase catalyzed oxidation of linoleate at 0°C. Using this value, it may be calculated that the constant ratio found by Lundberg and Chipault amounts to about 0.7 mols. of conjugated diene per mol. of peroxide.

On the basis of comparative studies of the autoxidation of methyl oleate and methyl linoleate Hilditch and co-workers (15,16,17,18) disputed the concept that the attack by oxygen occurs only at the alpha-methylenic positions. Hilditch (18) suggested a mechanism in which oxygen adds directly to the double bond, forming a transitory cyclic peroxide, which then may rearrange to a greater or lesser extent depending on experimental conditions to give hydroperoxides. Houtman and Orr (19) suggested a more precise formulation of the Hilditch concept in which it was postulated that, in lieu of a cyclic peroxide intermediate, a peroxidic diradical is formed by attachment of oxygen to only one of the carbons of the double bond. Although accepting this modification, Hilditch nevertheless indicates that in the autoxidation of oleates, particularly at high temperatures, many of the diradicals undergo ring closure so that cyclic peroxides are present in the autoxidation products.

THERE are two major points of difference between the formulation proposed by Hilditch and the alpha methylenic reactivity concept of Farmer, Bolland, and their co-workers. According to the former, a double bond shift must occur in all cases, even with a mono-olefin, if a hydroperoxide is the end product. The alpha methylenic reactivity concept, on the other hand, permits the formation of hydroperoxides with or without the shift of a double bond from its original position. That a double bond shift does occur even with mono-olefins has been shown by Swern *et al.* (20) in a study of the autoxidation of methyl oleate, but apparently the data were not unfavorable to either concept. A second point of difference is that the Hilditch formulation, even as modified by Houtman and Orr, does not offer a mechanism for the propagation of a chain reaction.

Subsequently, on the basis of the activation energies that are involved and other considerations, Farmer (4, 21), Bolland (12), and their co-workers have modified their findings to the extent that they postulate that the autoxidation chains are initiated by a reaction of the oxygen at the double bonds but are propagated by reactions at the alpha-methylenic positions. Hilditch (18) has accepted the concept of a chain mechanism but, by implication at least, apparently still allows for the possibility that even in the chain propagating reactions, oxygen may continue to add to the double bond although no mechanism has been proposed whereby this may occur.

Iodine values of various olefinic substances, including linoleates, have been found to fall during autoxidation to an extent that cannot be readily accounted for if it is assumed that all of the initial peroxides formed are hydroperoxides. Paschke and Wheeler (22), for example, studying the oxidation of the distilled esters of soybean oil acids, found a reduction in iodine value at low temperatures of oxidation which corresponded quite closely on a molar basis with the oxygen uptake. Only a minor part of the

apparent fall in iodine value could be accounted for by reaction between the peroxide and the iodide used in the iodine value determination.

On the other hand, Bolland and Gee (12) have stated that in the early stages of the oxidation of ethyl linoleate, hydroperoxides are formed quantitatively. This statement must apparently not be interpreted too literally since their formulation of a chain mechanism requires that reaction chains be terminated largely by an interaction of two peroxidic free radicals. Even if the statement is interpreted to mean that the peroxides formed in the early stages of the oxidation are all hydroperoxides, they appear to have given no conclusive supporting evidence. Moreover, on the basis of bond energies, they have indicated that theoretically a number of alternative reactions could occur which would yield various cyclic peroxides and polymeric peroxides that do not contain hydroperoxide groups.

Insofar as the autoxidation of linoleates is concerned, therefore, it may be said in summary that it appears to have been quite well established that the reaction proceeds via a free radical chain mechanism and that a high proportion of conjugated diene monohydroperoxides is produced whose hydroperoxide groups are attached to carbons in the 9 and 13 carbon positions. It may further be said that a complete understanding of the early stages of the reaction has by no means been achieved since a considerable number of unexplained and sometimes contradictory observations have been published. The two main points about which confusion still exists are 1. whether any appreciable amount of unconjugated diene hydroperoxides is formed and 2. whether any cyclic peroxides and polymeric peroxides are formed.

The data in this paper bearing on these two points are not as clear-cut as they might be, but on the basis of the experience gained in these initial experiments, it is believed that much more decisive data can be obtained which will indicate clearly the structures of most of the peroxides formed in the early stages of the autoxidation of methyl linoleate.

Experimental

The samples of methyl linoleate that were used were carefully prepared by a bromination-debromination procedure and exhibited analytical constants that agreed very closely with accepted values for pure methyl linoleate. The samples were oxidized by blowing air or oxygen through them at relatively low temperatures until peroxide values of 250 to 500 m.e./kg. had been attained. Concentrates of the peroxides were then prepared by fractionation methods that will be discussed in detail in a later publication. However, it may be said that the concentrates were obtained at low temperatures, and determinations of the peroxide values and spectral absorption at 2325 Å of the various fractions have indicated that the peroxides are not appreciably altered during their concentration, at least not so far as peroxide value and spectral absorption are concerned. The peroxide concentrates have not contained all of the peroxides that were formed and, in general, the concentration procedure has been found to isolate preferentially the more highly oxidized products of the oxidation.

Various investigators have attempted to learn something about the nature of fat peroxides by applying ordinary fat analytical methods such as determinations of iodine value, hydroxyl value, epoxy content, and other constants directly to the products of the oxidation of fatty substrates. Such techniques have generally been unsuccessful for the reason that many of the analytical methods give false values when applied to materials that contain peroxides.

TABLE I

Results of Analyses Applied Directly to Peroxides

Peroxide Concentrate No. 1	Assumed molecular weight, 326.5	
Peroxide value (13)	6268 m.e./kg. or 1.02 mols./mol.	
Spectral absorption, 2325 Å (13)	$a = 71.3$	M.E., 23,300 (1.48 mols. of C=C in conjugated form)
Woburn I. V. (23)	130.5	1.68 mols. of C=C/mol.
Wijs I. V., standard	131.7	1.69 mols. of C=C/mol.
Rapid Wijs I. V. (24)	122.6	1.58 mols. of C=C/mol
Epoxy oxygen (25)	2.88%	.59 mols./mol.
Hydroxyl oxygen (26)	34.2 mg. KOH/g.	.199 mols./mol.
Acid content	<1.0 mg. KOH/g.	$<.006$ mols./mol.

Table I shows the results of a few analyses applied directly to a concentrate of methyl linoleate peroxides. The peroxide content of this sample was 1.02 mols./mol. corresponding closely to the theoretical value for a methyl linoleate monoperoxide. The molecular absorption at 2325 Å, 23,300, indicates that there was a considerable amount of conjugated diene unsaturation in this peroxide concentrate. Using Bergström and Holman's value of 31,400 for the molecular extinction of conjugated diene peroxides of linoleate the absorption in this sample corresponds to about 0.74 mols. of conjugated diene per mol. of the original methyl linoleate. Multiplying by 2, the quantity of double bonds in a conjugated condition must be approximately 1.48 mols./mol. of the original methyl linoleate.

This figure is only slightly less than the total content of double bonds indicated by determinations of the iodine value by three methods. These iodine values, if taken at face value, would indicate that there was a low content of unconjugated double bonds. Moreover, it should be observed that the Wijs method actually gives the same value as the Woburn method, which is unexpected in view of the fact that the Wijs method is known to give low values with conjugated substances.

Although Farmer and Sundralingam (1) have found that the hydroperoxide of cyclohexene gives an iodine value that is only about 6% below the expected value, the comparisons that have just been made suggest that the iodine values on linoleate peroxides are not trustworthy. It will also be shown later that when the peroxide groups are reduced, the reduction products show a considerably higher iodine value, which cannot be accounted for by double bond regeneration during the reduction. Hence it is concluded that in this case the peroxide groups interfere at least to some extent with the determination of the iodine value.

An analysis of the epoxy oxygen content gave a very high value, amounting to 0.59 mols. of epoxy oxygen per mol. of original linoleate. This value cannot be reconciled with any of the other data that have been obtained by others in studies of the oxidation of linoleates nor can it be reconciled with the data that will be discussed. It is therefore believed that this is a spurious value arising from a rearrange-

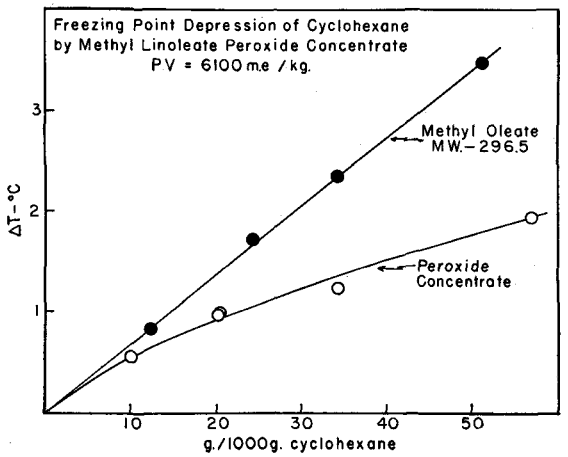

FIG. 1.

ment of the peroxide during the analytical determination. It should be pointed out that a mineral acid is used in this determination, and as Farmer (4) and others have indicated, when an olefin hydroperoxide is treated with mineral acids, the hydroperoxide group oxidizes an adjacent double bond, apparently forming first a hydroxy epoxy compound and ultimately a trihydroxy compound.

A determination of the hydroxyl oxygen indicated the presence of 0.199 mols./mol. in the peroxide concentrate. This analytical determination involved the use of acetic anhydride, and in this case also the conditions of the determination may have caused the peroxide group to oxidize adjacent double bonds to some extent.

The free fatty acid value was found to be very low (0.006 mols./mol.) and this low value was anticipated since there is no evidence to indicate that any appreciable hydrolysis occurs during the early stages of the autoxidation of fatty acid esters. The determination of free carboxyl groups by titration with alkali is probably not affected by the presence of peroxide groups.

The application of some of these analytical methods directly to materials containing peroxides, then, offers no hope of a quantitative determination of the structure of the peroxides, and other techniques are evidently necessary.

Bergström (8), in his study of the oxidation of methyl linoleate, found that the peroxides in the oxidized substrate could be completely reduced in aqueous ethanol by sodium sulfite. He reported that the ultraviolet absorption at 2320 Å remained unchanged in this reduction. It was therefore decided to attempt the reduction of a peroxide concentrate by addition of potassium iodide to a chloroform acetic acid solution of the peroxide concentrate, essentially under the same conditions that are used in the determination of peroxide values. It was found that in this reduction also the ultraviolet absorption at 2325 Å remained almost unchanged on a molar basis, in some cases showing no change, and in other cases showing a very slight increase.

These observations appeared to offer a method for learning something about the structure of the peroxides because if it could be shown that the double bonds were substantially unaffected by the reduction, then by reducing the interfering peroxide groups it might be possible to apply some of the common fat analytical methods to the reduced products to determine to some extent the structure of the original peroxides. It is on this basis that current studies in this laboratory are being made.

First, results of some studies of the molecular weights of the peroxide concentrates and of the products obtained by reduction of the concentrates will be considered. Attempts were first made to determine molecular weights by a cryoscopic method using cyclohexane as the solvent. Figure 1 shows a plot of the observed freezing point depressions against concentration for methyl oleate and for a methyl linoleate peroxide concentrate. It is apparent from the curve for the peroxide concentrate that this material does not form an ideal solution in cyclohexane, probably because of association of the molecules at higher concentrations. Cyclohexane was therefore regarded as an unsatisfactory solvent for the determination of the average molecular weight of the peroxides.

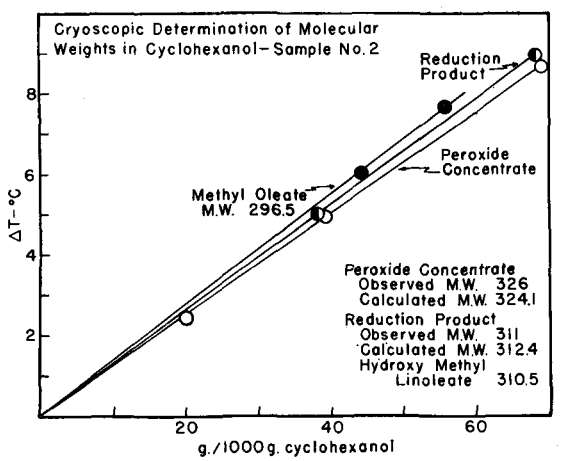

FIG. 2.

Figure 2 shows results that were obtained when cyclohexanol, carefully purified by distillation, was used for the molecular weight determinations. In this case methyl oleate, methyl linoleate peroxide concentrate, and products of reduction of the concentrate all gave straight lines which when extrapolated passed through the origin. Highly purified methyl oleate was used as the standard in determining the molecular freezing point depression for cyclohexanol.

Assuming that all of the nonperoxidic constituents of the peroxide concentrate are methyl linoleate, the calculated molecular weight of a peroxide concentrate containing .927 mols. of monomeric peroxide per mol. is calculated to be 324.1, and the value that we have observed is 326.

In the reduced concentrate certain corrections and assumptions are necessary in order to obtain a calculated value for the molecular weight. It was found for example that some of the hydroxyl groups formed in the reduction of peroxide had been acetylated during the reduction procedure. It was also found that the ester linkage at the end of the fatty acid chain had been hydrolyzed to some extent. After making corrections based on the acid value, hydroxyl value, and acetyl content of the reduced product, and assuming that any other materials present in the reduced

product consisted entirely of methyl linoleate, the molecular weight for a monomeric reduction product was calculated to be 312.4. Actually this value turned out to be not greatly different from the theoretical value for a pure methyl hydroxy-octadecadienoate, the value for the latter being 310.5. The observed value, 311, agrees with both of these values, and similar observations on other samples are interpreted to mean that in this concentrate virtually all the peroxides were monomeric and that their reduced products were also monomeric.

This concentrate was obtained from a batch of methyl linoleate that was oxidized to a peroxide value of 487 m.e./kg. at 40°C., and the concentrate contained about 33% of the original peroxides. The remaining 67% was not destroyed but simply lost during the concentration process. In other cases much higher recoveries of the peroxides were made, and the results of molecular weight determinations and other values to be discussed were in the main not significantly different.

Before going on to a consideration of other data obtained in this sample, it should be said that it is unfortunate that data for this sample must be presented rather than for others that have been studied because this peroxide concentrate had a somewhat lower peroxide content than might be desired and because several other factors associated with the preparation, such as the low recovery and factors associated with the reduction, make quantitative calculations difficult. It seems best to consider this sample at this time, however, since it is the one on which the most complete data are available.

It should be emphasized also that some of the data that will be presented will be subject to error for another reason. In previous work it was found that some of the oxygen absorbed by methyl linoleate under these conditions forms conjugated diene ketones and also other oxygenated substances, which do not analyze as peroxides by the iodometric method (13). In another oxidation of methyl linoleate to a peroxide value of 500 m.e./kg. at 40°C., 15.4% of the total absorbed oxygen was utilized in the production of such substances. A completely quantitative analysis of the following data is therefore not possible.

Some discrepancies attributable at least in part to these factors are apparent in the results of carbon, hydrogen, and oxygen analyses which are shown in Table II. Three sets of values are given for both the peroxide concentrate and its reduced products.

In the case of the peroxide concentrate the first set of values was determined by analysis. The second set consists of the calculated values for a pure methyl linoleate monoperoxide. The third set was calculated assuming that the peroxide concentrate contained methyl linoleate peroxides in the amount indicated by the peroxide determination and that the remaining materials consisted entirely of methyl linoleate. The important values to be considered are those for the oxygen content. It is seen that the observed oxygen content, which is subject to some error since it is obtained by difference, is about 1.3 percentage points higher on the total matter basis than the value calculated from the observed peroxide content. This means that the observed oxygen content is actually about 7% higher than the calculated. Since one may calculate from the molecular weight of the peroxide concentrate, that about 9.85% of the

TABLE II

Observed and Calculated Values for Carbon, Hydrogen, and Oxygen Content
(Peroxide Concentrate No. 2 and Reduction Product)

	Carbon	Hydrogen	Oxygen (by difference)
Peroxide Concentrate			
Found	% 69.08	% 10.63	% 20.29
Calculated for pure methyl linoleate monoperoxide	69.90	10.50	19.60
Calculated, based on observed peroxide content	70.45	10.58	18.97
Reduction Product			
Found	71.60	10.73	17.67
Calculated for pure methyl hydroxy-linoleate	73.50	11.04	15.46
Calculated, based on other analytical data	73.75	11.05	15.48

total matter consists of oxygen in the carboxy group, the analytically determined oxygen in locations other than the carboxy group is about 14.5% higher than calculated. Part of this discrepancy can be accounted for by the ketones and possibly aldehydes that are formed concurrently with the peroxides in the early stages of the oxidation. Part of it may also conceivably be accounted for by the formation of very stable peroxides that are not reduced by the iodometric method. Actually, the discrepancy of 14.5% is slightly less than the 15.4% difference between absorbed oxygen and peroxidic oxygen that has been observed in other samples oxidized under similar conditions. These high values for the oxygen content of the peroxide concentrate have been consistently obtained in all samples.

In the case of the reduced product the first values are again those that were determined experimentally. The second set consists of values calculated for a pure methyl hydroxy-octadecadienoate; the third set of values is calculated on the basis of determinations of the acid value, acetyl content, hydroxyl value, and molecular weight of the reduced peroxides. The observed oxygen content is about 2.2 percentage points higher than either of the two calculated values. However, the second calculated value is certainly subject to considerable error since it contains all the accumulated errors encountered in the analyses of the acid value, acetyl content, hydroxyl value, and molecular weight. All that may be concluded from these carbon, hydrogen, and oxygen analyses is that the peroxide concentrates contain oxygenated materials that are not reduced by the iodometric method and also that some of the peroxides may be reduced without losing oxygen. The latter is a possibility, for example, if cyclic peroxides are present which may be reduced to give two hydroxyl groups. The probable presence of some alpha glycolic groups in the reduced products suggests that such reduction actually does occur to a minor extent.

Table III shows additional data obtained on the same sample of peroxide concentrate and its reduction product. The molecular absorption at 2325 Å calculated on the basis of the observed molecular weight was found to be 22,700 in the peroxide concentrate. In the reduced peroxide the molecular absorption at 2325 Å was 23,900, a slight increase. The reason for the increase is not known. It is not unreasonable to suppose, however, that the magnitude of the absorption by conjugated linoleate hydroperoxide and the corresponding conjugated hydroxy

TABLE III

Additional Analytical Results Obtained for Samples Considered in Table II

(Peroxide Concentrate No. 2 and Reduction Product)

	Peroxide concentrate	Reduction product
Observed M. W.	326 (calc. 324.1)	311 (calc. 312.4)
Peroxide content (13)	.927 mols./mol.	<.01 mols./mol.
Molecular absorption, 2325 Å (13)	22,700	23,900
Molecular absorption, 2775 Å (13)	84.5	728
Woburn I. V. (23)	132.5 (?)	173.6 (2.12 C=C per mol.)
Acid content	.....	.058 mols./mol.
Alpha-glycolic content (27)	.....	.067 mols./mol.
Saponification equivalent	.....	288.6
Hydroxyl content, observed (26)	.....	.852 mols./mol.
Hydroxyl content, corrected by acetyl content	.....	.930 mols./mol.
Content of monohydroxyl plus alpha-glycolic groups	.....	.863 mols./mol.
Epoxy content (25)	.....	(0 mols./mol.)[1]

[1] Value observed in other samples.

linoleate may be slightly different by this amount. The difference could also mean that a small amount of additional conjugated diene was somehow formed during the reduction. In any case it seems permissible to hypothesize that the conjugated systems present in the peroxides are almost unaffected by the reduction reaction.

Moreover, the Woburn iodine value of the reduced compound based on the observed molecular weight corresponds to 2.12 double bonds per mol. The variation from the anticipated value of 2.00, assuming that there had been no change in the number of double bonds present in the original methyl linoleate, is well within the limits of experimental error, particularly if one takes into account the small amount of triene or conjugated diene ketone that appears to have been formed as indicated by the absorption at 2775 Å. The iodine value suggests therefore that in the main most of the double bonds either remain undestroyed even though some of them are shifted during the peroxide formation or that they are regenerated during the reduction. The latter possibility appears to be rather unlikely, particularly since no appreciable increase in the amount of conjugated diene occurs. If double bonds are regenerated, they must necessarily be unconjugated, and there is no apparent mechanism by which such unconjugated double bonds could be regenerated in these systems.

On the reduced peroxide, determinations have also been made of the acid value by titration with alkali, the alpha glycolic value by titration with periodic acid, and the saponification value and hydroxyl value by standard methods.

The acid value in this sample, although still low, was unfortunately relatively high compared with other samples, being .058 mols. per mol., due apparently to hydrolysis resulting from the addition of a small amount of water during the reduction of this sample. In other samples of peroxide concentrates reduced under slightly different conditions, for which complete data were not obtained, the acid value was much less, in one case being entirely negligible.

The alpha glycolic value was also considerably higher than in the other samples, in this case being .067 mols. per mol., which, however, is still quite low. It should be pointed out, moreover, that this must be regarded as a maximum value since the periodic acid used in the titration is known to react with some other compounds in which oxygen is attached to adjacent carbons, for example, alpha ketol groups. It is interesting to speculate on the possible reasons for the higher alpha glycolic value obtained in this case. It seems entirely reasonable to expect that during the oxidation of methyl linoleate some diperoxides would be formed having some such structure as is shown in Figure 3. It has been observed frequently in

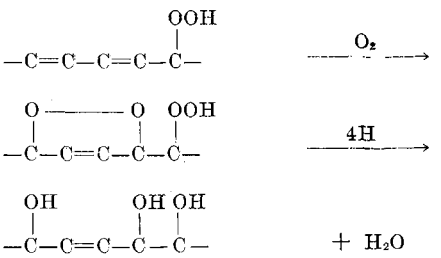

FIG. 3. A possible diperoxide and reduction product.

this laboratory that the peroxide concentrates are extremely susceptible to further oxidation and upon brief exposure to air readily yield materials with peroxide values of 8000 m.e./kg. or more.

One would expect that a conjugated linoleate hydroperoxide would be readily susceptible to further oxidation of the conjugated diene system. Peculiarly, this further oxidation, although it occurs readily in the peroxide concentrate, does not occur readily when the peroxide is diluted with unoxidized methyl linoleate. Apparently the methyl linoleate protects the conjugated monohydroperoxide from oxidation to a considerable extent, possibly through some association mechanism, thus minimizing the formation of diperoxides.

A likely explanation of the alpha glycolic value therefore is that some diperoxides were formed during the oxidation of methyl linoleate. The somewhat higher alpha glycolic value obtained in this sample as compared with other samples can then be accounted for on the basis that only 33% of the total peroxides was isolated during the preparation of this peroxide concentrate. Since this method of concentration favors the isolation of the more highly oxygenated materials, a greater proportion of diperoxides may have been isolated.

It is noteworthy that even in this sample the alpha glycolic content is much less than the .2 mols./mol. found by Bergström in the products obtained by hydrogenation of samples of methyl linoleate that had been oxidized under conditions comparable to those used here. The data on this and other samples are strong evidence that most of the alpha glycolic content found by Bergström was produced during hydrogenation through some rearrangement reaction, and, as will be indicated later, this may also account for his failure to find the 11-hydroxystearate in the hydrogenated products.

Returning to a consideration of Table III, the saponification equivalent was found to be 288 whereas the molecular weight of the reduced compound was 311. This indicated that some of the hydroxyl groups were acetylated during the reduction and, assuming that the entire difference is due to acetylation, it may be calculated that the amount of hydroxyl groups acetylated was approximately .078 mols./mol.

The observed hydroxyl content of the reduction product corrected for free acid was .852 mols./mol. If this value is corrected by the acetyl content of the reduced compound, the hydroxyl content in the absence of acetylation would be .930 mols./mol. This corresponds very closely with the value of .927 mols./mol. anticipated from the peroxide value, assuming that one peroxide group yields one hydroxyl group on reduction.

If, however, the alpha glycolic content is considered to be a measure of the amount of peroxide that on reduction yields two hydroxyl groups for each peroxide group, then the original content of peroxide calculated from the corrected hydroxyl content and alpha glycolic content would be .863 mols./mol., about 7% lower than the observed peroxide content. While the agreement here is not as good, it nevertheless does not rule out the possibility that the diperoxides are reduced in the manner suggested in Figure 3.

The epoxy content of this sample was not determined, but it is indicated in parentheses that it was probably 0 mols./mol. since this is the value that was obtained on other samples of reduced peroxide. It is thus indicated that the hydroperoxides and cyclic peroxides, if present, are not reduced to epoxy compounds by the method of reduction that has been used.

Summary

A technique for studying the early stages of the oxidation of methyl linoleate has been discussed. It is suggested now that this technique may be applied to advantage in studying the structures of the compounds formed in the drying of drying oils, at least in the early stages. In such studies the oxidation would be carried to sufficiently high levels so that the isolation of the oxidized products would not be necessary in order to conduct accurate analytical determinations. For such studies it would also be desirable to devise suitable analytical methods for the determination of ether linkages and carbonyl groups.

These preliminary studies have shown that the initial products of the oxidation are monomeric. Determinations of the spectral absorption, Woburn iodine value, hydroxyl content, and other values suggest that most of the initial oxidation products are monomeric monohydroperoxides, most of which are conjugated. Since the corrected hydroxyl value of the reduced peroxides corresponds closely to the peroxide content of the peroxide concentrate and since the content of the conjugated diene is considerably less than the content of peroxide, it is clearly evident that unconjugated hydroperoxides are also formed, possibly mainly the 11-hydroperoxide. Bergström's failure to find 11-hydroxystearate or any other monohydroxystearate except 9- and 13-hydroxystearates in the hydrogenated products of oxidized linoleate is therefore due to secondary reactions during hydrogenation in the course of which alpha glycolic groups are formed.

In addition, this study indicates that diperoxides are formed to some extent, possibly by a 1,4-addition of oxygen to the 9- and 13-conjugated monohydroperoxides. It is indicated that the second peroxide group is cyclic. The data also show that during the oxidation other oxygenated products are formed in appreciable amounts which are not reduced by potassium iodide in glacial acetic acid and chloroform.

Acknowledgment

We wish to acknowledge our indebtedness to D. H. Wheeler and the General Mills Research Laboratories for their assistance and cooperation in making a number of the analytical determinations, particularly the carbon, hydrogen, and oxygen analyses and the Woburn iodine value determinations.

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