A STUDY OF THE REACTION OF METHYL LINOLEATE WITH MOLECULAR OXYGEN

by

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INTRODUCTION

Atmospheric oxygen is the most prevalent as well as economically important oxidizing agent for fats and fatty acids. Its action may be beneficial or deleterious depending on the conditions and circumstances under which it occurs. For example, the process of film formation in applied protective coatings is essentially an oxidation process. The role of oxygen in the initial formation of the film is beneficial but prolonged and excessive oxidation of the film will result in its ultimate failure. Because this failure leads to serious economic losses many studies and a vast amount of literature have accumulated on the subject. Although the last decade has seen notable advances in our knowledge of the mechanism of the autoxidation of many olefins, including such technically important substances as fats, drying oils and rubber, as yet there is no clear understanding of the fundamental reactions involved.

It has long been known that peroxides are formed in the early stages of autoxidation of olefins and in accordance with the Engler and Bach hypothesis (6) it has usually been assumed that a molecule of oxygen added at the double bond to form a peroxidized compound which in turn could oxidize other oxidizable material. In 1909 Fokin (10) proposed a theory in which the first step in the autoxidation of an ethylenic bond was presumed to occur thru the formation of an ethylene oxide ring.

$$-CH=CH- + \tfrac{1}{2}O_2 \rightarrow -CH\!-\!\underset{O}{\diagup\diagdown}\!-CH-$$

Pokin arrived at this conclusion from a study of the unsaturated fatty acids in the presence of several metal catalysts and considered the data from a kinetic standpoint.

Staudinger (20) proposed a theory of autoxidation based on the assumption that the peroxide proposed by Bach and Engler was not the first but the second step in the autoxidation of ethylenic bonds. Staudinger based his conclusions on a study of asym-diphenylethylene. Diphenylethylene peroxide was isolated and found to be stable, but the product of first addition of oxygen could not be isolated and exploded when heated to 40° - 50°. He proposed the term mol-oxide for the primary oxidation product, reserving the peroxide term for compounds of known structure.

In 1936, Criegee (4) suggested that cyclohexene autoxidized to form a hydroperoxide having an intact double bond. Later work (5) proved the structure of the primary oxidation product of cyclohexene as being a hydroperoxide because the material could be reduced to the cyclohexenol, will absorb one mol of bromine and when oxidized with strong oxidizing agents, give a hydroxy adipic acid as the main product. Rieche (17), in a discussion of oxidation reactions, suggested in many cases O_2 does not react with the double bond (in contrast with O_3) but enters between a C - H bond.

$$R\text{-}CH=CH\text{-}R + O_2 \rightarrow R\text{-}CH=\underset{\underset{OH}{|}}{\overset{\overset{O}{|}}{C}}\text{-}R$$

This suggestion was the foundation for the work of Farmer and co-workers. Farmer and Sutton (9) by oxidizing methyl

oleate with atmospheric oxyhen and carefully fractionating the products at temperatures below 100° and pressures down to 10^{-5} mm obtained a fairly pure unsaturated hydroperoxide of methyl oleate. Swift, Dolleer and O'Conner (23) by low temperature crystallization of oxidized methyl oleate obtained the same unsaturated hydroperoxide as Farmer but at about 95 percent purity. The hydroperoxide gave a mixture of 8 and 11 hydroxy stearic acid after reaction with 2 mols of hydrogen. It added iodine to give a theoretical iodine number, was reduced by sodium sulfite to the unsaturated hydroxy compound and gave an almost theoretical peroxide number when reacted with HI. From these data two structures were assigned to the methyl hydroperoxide oleate.

$$CH_3(CH_2)_7CH{=}CH{-}CHOOH{-}(CH_2)_6COOCH_3$$

$$CH_3(CH_2)_6CHOOH{-}CH{=}CH{-}(CH_2)_7COOCH_3$$

From this work Farmer came to the conclusion that the point of oxygen attack in autoxidation reactions is at the methylene group adjacent to the double bond.

In 1942, Farmer (7) had pointed out that this methylenic reactivity was typical of the reactions with olefins of free phenyl radicals produced from dibenzoyl peroxide and other materials. He suggested therefore that autoxidation involved consecutive dehydrogenation and oxygen addition processes in a radical-chain raction sequence in which the chain starting catalyst .R has the nature of a free radical and could be .OOH.

$$-\underset{H}{CH}-CH=CH+\dot{R} \rightarrow -\dot{C}H-\underset{H}{C}=\underset{H}{C}+RH$$

$$-\dot{C}H-CH=CH+O=O \rightarrow -\underset{O-O\cdot}{CH}-CH=CH-$$

$$-\underset{OO\cdot}{CH}-CH=CH- + CH_2-CH=CH- \qquad CH-CH=CH + \dot{C}H-\underset{OOH}{CH}-CH-$$

This methylenic activity is ordinarily unknown among the reactions of olefins. However, Rust and Vaughn (19) have shown that at high temperatures, chlorine will substitute in the allyl position in an olefin giving the unsaturated chlorinated compound. Bromine is also easily substituted into the active methylene group in malonic ester. In this case the methylene group is considered to be activated by the carboxyl groups. Kummerow and Green (14) isolated material containing bromine from B-linoleic acid which was believed to contain some 11 bromo linoleic acid. These reactions indicate that the methylene group in some olefins is sufficiently activated to enter into a reaction with reagents such as the halogens.

One essential feature of the a-methylenic dehydrogenation of olefins, such as oleic acid, is the fact that it yields two forms of the radical having the double bond between different carbons with a shift also of the unpaired electron.

$$A-\dot{C}H-CH=CH-B \text{ and } A-CH=CH-\dot{C}H-B$$

This mesomerism of course stabilizes these radicals, and is in all probability, the essential structural feature which reduces the activation energy of the primary dehydrogenation process to a value which is sufficiently below that for

hydrogen abstraction from a saturated paraffin chain to allow the autoxidation reaction to occur with reasonable speed at moderate temperatures. Because of this mesomeric shift of the double bonds and the free electron two possible hydroperoxides may be formed from a mono olefin. Atherton and Hilditch (1) have confirmed this by isolating from the products of autoxidation of methyl oleate at 20°, two dibasic acids, suberic and azelaic, and two monobasic acids, octoic and nonanoic, thus showing that oxidation can occur at carbon atoms 8 and 11 of the chain as well as at the double bond between carbon atoms 9 and 10.

The work of Farmers and others in isolating a moderately pure peroxidized methyl oleate was a fundamental advance and stimulated work on the chemistry of autoxidation. The work was extended to the more highly unsaturated acids such as linoleic and linolenic acids. Bolland and Koch (3) studied the reaction of ethyl linoleate with oxygen and found that all oxygen up to an intake below 4 percent could be demonstrated as hydroperoxide groups. By chromatagraphic absorption of a known weight of peroxidized material, followed by weighing of the unoxidized ester in the eluate, it was shown that only one hydroperoxide group is present in each of the oxidized molecules. The ultra violet spectra were also examined and it was found that a strong absorption band existed at 2315A. This ultra violet absorption band is characteristic of conjugated dienes and by calculations, they arrived at the conclusion that 70 to 85 percent of the linoleate

oxidation product consists of conjugated isomers. On the basis of these results it was concluded that the oxidation of ethyl linoleate was a free radical mechanism. The oxidative attack at the active methylene group of (I) results in the formation of a free radical II, which is a resonance hybrid composed of three equivalent structures. The hydroperoxidic product III derived from the radicals would then be expected to be composed of the three isomeric forms with 67 percent conjugation occuring.

$$CH_3(CH_2)_4\ CH=CHCH_2\ CH=CH\ (CH_2)_7\ COOE$$

I

$$CHR_1=CH-CH=\overset{*}{C}H-CHCHR_2 \qquad CHR_1=CH-CH=CH-CHOOHR_2$$

$$CHR_1=CH-\overset{*}{C}H-CH=CHR_2 \qquad CHR_2=CH-CHOOH-CH=CHR_2$$

$$\overset{*}{C}HR_1-CH=CH-CH=CHR_2 \qquad CHR_1\ OOH-CH=CH-CH=CHR_2$$

II III

This was in agreement with Farmers theory of the reaction mechanism.

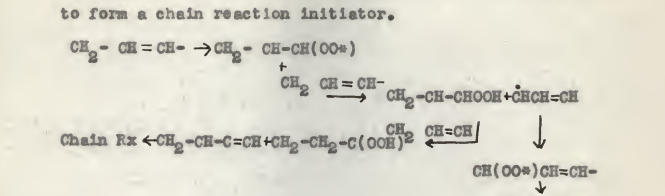
This reaction was studied further by Gunstone and Hilditch (11) who oxidized samples of methyl linoleate at different temperatures, determined the iodine number, peroxide number and percent of conjugated material at different stages of oxidation. It was found that seemingly different reactions occurred at higher temperatures but at moderate temperatures the hydroperoxide was formed. The iodine numbers indicated that double bonds were being saturated but no correlation existed between the peroxide valve and decrease in iodine number.

Bergstrom (2) confirmed Farmer's theory by oxidizing methyl linoleate with molecular oxygen at 37°. It was found that all oxygen was present as peroxide group up to .2-.3 mol O_2 per mol of methyl linoleate. This partially oxidized material was then directly hydrogenated using a platinum oxide catalyst. The hydrogenated product was separated chromatagraphically into various fractions. Two of these fractions were thought to be the 9 hydroxy stearic acid and the 13 hydroxy stearic on the basis of mixed melting points. No 11 hydroxy stearic acid was found. These findings lend support to the theory of Bolland and Koch (3) that a free radical is formed with consequent shift of the double bond to a conjugated system and a hydroperoxide group is attached after the conjugation occurs. The absence of the 11 hydroxy stearic acid in the hydrogenated mixture suggests that if the 11 hydroperoxide methyl linoleate is formed, either the acid was overlooked in the chromatagraphic separations or the 11 hydroperoxide compound is very unstable and rearranges to a more stable isomer on catalytic hydrogenation.

Having established the identity of the first reaction product as a hydroperoxide, attention was given by Farmer and co-workers to the problem of the mechanism by which oxygen molecules are incorporated into the oxidized molecule and to the problem of peroxide breakdown.

Farmer, in a discussion of autoxidation reactions, (8) was of the opinion that the initiation of the autoxidation

reaction was due to the addition of oxygen at a double bond to form a chain reaction initiator.



As thus shown, the amount of actual addition at the double bond would be relatively small but would start the necessary reaction chains. Supposedly, the energy requirement for the addition at a double bond is smaller than the 80Kcal necessary to dehydrogenate an active methylene group.

Peroxide breakdown is one of the secondary reactions in autoxidation and has, as yet, not been investigated to any great extent. The hydroperoxide group on one molecule may oxidize another unsaturated molecule to give several products as shown by Swift and Dollear (21) or it may break the carbon chain to which it is attacked to give aldehydes. This was shown by Swift, et al., (22) who decomposed pure methyl hydroperoxide oleate thermally and was able to isolate and identify 2- undecanal from the decomposition products. The polymeric tendency in peroxidation is great in conjugated olefins but does not disappear entirely in the case of mono-olefins and isolated systems of double bonds. Polymerized products are always obtained when the products of autoxidation are separated. Very little work has been done on these

polymers and as yet it is not known what type of linkages are present in the polymers. The study of the secondary reactions of autoxidation is very difficult because of the great variety of products obtained under different conditions. These reactions are being studied however and a complete solution to the problem should not be too far in the future.

Although the literature on the autoxidation reaction is voluminous and filled with different theories and contradictions, this report is an attempt to add to the understanding of the reaction by a careful quantitative study of the reaction of methyl linoleate with molecular oxygen.

EXPERIMENTAL

Preparation of Materials

Unless otherwise indicated, the linoleic acid used in these experiments was obtained from the methyl ester which was prepared by the debromination of tetrabromostearic acid by the Rollett method (18).

Preparation of the Fatty Acid. Three hundred g of potassium hydroxide were placed in a five-liter round-bottom flask, 1200 ml of ethyl alcohol and 100 ml of water were added, and the mixture was heated to near boiling on a steam bath. One thousand grams of cottonseed oil were added to the hot solution, condenser attached, and refluxing allowed to proceed for 30 minutes. The hydrolysate was cooled to about 40° C in a stream of tap water and 1300 ml of cold distilled water added. With continued cooling and agitation, 550 ml of cold concentrated hydrochloric acid were added in small portions. The cold mixture was then transferred to a large separatory funnel and shaken vigorously to insure complete decomposition of the soaps. The fatty acids were washed twice with about one liter of distilled water, care being taken to avoid emulsification. One liter of redistilled petroleum ether was added and the fatty acids were washed again. After the water had been drawn off the fatty acids were placed in a five-liter round-bottom flask to which about 50 g of anhydrous sodium sulfate were added. One liter of redistilled petroleum ether was then added and the

solution allowed to stand overnight at -5° C. The saturated acids and the sodium sulfate were filtered off and one liter of redistilled petroleum ether was added to the filtrate which was then ready for bromination.

Preparation of Crystalline Tetrabromostearic Acid. The five-liter flask containing the solution of fatty acids was clamped firmly into an ice-salt bath, the flask being at least three inches above the bottom of the bath to provide proper cooling. A mechanical stirrer with sufficient speed and power to produce good mixing was adjusted to about one-half inch from the bottom of the flask. Bromine was added from a separatory funnel at such a rate that the temperature of the reaction mixture at no time exceeded 10°. About 580 g of bromine were required for complete saturation, as indicated by the persistence of a bromine color. The flask was corked tightly and allowed to stand overnight at -5°. The crystalline crude tetrabromostearic acid was collected on a Buchner funnel, washed with redistilled petroleum ether and transferred to a dry five-liter round-bottom flask. Five liters of redistilled petroleum ether and 1.5 liters of ether, or more if necessary to effect complete solution, were added and the tetrabromostearic acid was brought into solution by refluxing on a steam bath. Twenty g of Norit were added, refluxing continued for a few minutes, and then the hot solution was filtered through a warm Buchner funnel. Oversized filter paper was used in the funnel and was kept firmly in place by a water bath ring which fitted snugly in

the funnel. The filtrate was allowed to stand overnight at -5°. The product was filtered on a Buchner funnel and washed with redistilled petroleum ether. The white crystals were dried at room temperature and their melting point determined (114-115°). If the melting point was low, the product was recrystallized from petroleum ether before proceeding.

Preparation of Methyl Linolate. Two hundred g of tetrabromostearic acid and 200 g of granular zinc were mixed together and placed in a dry, ground-neck, round-bottom flask. A condenser was attached and 200 ml of methyl alcohol were added and the acid dissolved by warming carefully on a steam bath. Cooling in a stream of tap water was necessary to control the initial reaction, after which the mixture was allowed to reflux for two hours on the steam bath. The reaction mixture was cooled and poured into a separatory funnel containing 500 ml of distilled water. A small amount of hydrochloric acid was added to decompose any zinc soaps which might be present and the mixture was shaken vigorously and allowed to stand until the ester had separated completely. The aqueous phase was then drawn off and extracted twice with 200 ml of petroleum ether. The ester and ether solution was then washed with 500 ml of 2 percent sodium carbonate solution and twice with 100 ml of cold water. The washed ether solution was dried over sodium sulfate, filtered and the solvent removed by means of a water pump. The ester was distilled in an all glass vacuum still and the fraction collected that boiled at 135°-140°, .1mm pressure.

Preparation of Linoleic Acid. The ester (200 g) was placed in a five-liter round-bottom flask and saponified with 1200 ml of 5 percent alcoholic potassium hydroxide by standing overnight at room temperature. An equal volume of distilled water was added and the mixture extracted twice with one-half volumes of redistilled petroleum ether to remove any unsaponified ester. The saponified portion was then acidified with dilute hydrochloric acid (1:1) while cooling and shaking vigorously. The linoleic acid was extracted with an equal volume of petroleum ether and washed with equal volumes of 50 percent alcohol and water and dried with sodium sulfate. The solvent was removed under reduced pressure with a water pump and the acid distilled under a pressure of .1 mm. The portion which boiled at 150°-155° was collected and used immediately or preserved by sealing in 15 ml glass bulbs under a high vacuum.

Preparation of Δ^{10-12} Octadecadienoic Acid. The conjugated linoleic acid was prepared by the method described by vonMikusch (25).

Two hundred grams of dehydrated castor oil was saponified in a solution of 10 g potassium hydroxide, 240 ml of ethyl alcohol and 20 ml water. After saponification was complete the solution was acidified with hydrochloric acid and the fatty acids extracted with Skelly solve F, washed with water, dried over sodium sulfate and the solvent removed under a vacuum. The fatty acids were then isomerized

by dropping them slowly into a mixture of 400 g potassium hydroxide and 1 liter of ethylene glycal heated to 180°. After all the fatty acids had been added the solution was heated for ½ hour while keeping the solution covered with nitrogen gas. After the isomerization was completed, the hot solution was poured over 1000 g chipped ice, acidified with hydrochloric acid and the fatty acids extracted with Skelly solve F, washed with water, dried over sodium sulfate and the acids allowed to crystallize from the petroleum ether solution by cooling to -10° for 48 hours. The solid acid was filtered off, crystallized again from Skelly solve F, and twice from ethyl alcohol. This yielded Δ^{10-12} octadecadienoic acid of about 98 percent purity.

Determination of Constants

Hydrogen Number. The apparatus used for the determination of the hydrogen numbers (Fig.1) was a modification of the apparatus described by Johns (12). The bulb of the reaction flask was made from a 100 ml Kjeldahl flask by sealing a ground glass connection to the shortened neck of the flask. A short stop cork was sealed into the neck of the flask. Also in the neck of the flask, about 2 cm below the lower portion of the ground glass connection, a small bore tube was sealed, fitted into this and extending into the neck of the flask was a glass rod of a diameter to just fit smoothly in the tube but yet free to move. The glass rod

was connected to the outside tube by a short piece of rubber tubing fitted over both to make an air tight connection. This "trigger" served to hold the tube containing the sample in the neck of the flask until the catalyst and solvent had been saturated with hydrogen. A capillary tube lead from the reaction flask to the measuring burette, the connection between the flask and capillary was made by means of ground glass connections. The measuring burette was a pyrex 25 ml burette with the stop cock removed. The capillary leading from the reaction flask was sealed to the top of the burette. A T joint was sealed to the bottom of the burette, one arm lead to a leveling bulb by means of rubber tubing and the third arm lead to a tube the same length and diameter as the measuring burette. This tube served as an inlet tube for the hydrogen when connected to the hydrogen source and also as a monometer for accurate leveling of the burette liquid. The whole apparatus was clamped to a board. Two such pieces of apparatus were constructed and mounted on boards which were pivoted on a single support. The apparatus was agitated by connection to a motor driven eccentric. A vibration rate of about 150 per minute was used and controlled by a variable resistance connected to the motor.

The catalyst, a small amount of platinum on zirconium oxide was introduced into the reaction flask and 10 cc of ethyl alcohol then added as a solvent. The sample, 50 to 100 mg was weighed into a thin walled, flat bottomed glass capsule. These capsules were made from thin walled test

tubes and were 1 cm in diameter and about 2 cm long. This capsule was placed in the neck of the reaction flask and supported by the glass rod "trigger". The stop cock in the flask was opened. The reaction flask was connected to the apparatus through the carefully greased ground glass connection and clamped securely in place. The mercury in the burette was lowered below the bottom of the burette and hydrogen allowed to sweep through the apparatus for several minutes. Before the hydrogen entered the apparatus it was bubbled through a flask containing ethyl alcohol. This procedure reduced to a minimum the time necessary for the saturation of the atmosphere in the apparatus. When the apparatus had been thoroughly swept out, the stop cock was closed, the mercury level raised to the lower mark on the burette, the source of hydrogen disconnected and the agitation of the apparatus started, still retaining the sample tube in the neck of the flask. When the catalyst and alcohol had been saturated with hydrogen as indicated by a constant volume reading of the burette, the reading of the burette, temperature and barometric pressure were noted. The sample tube was dropped into the reaction flask by pulling the glass rod trigger outward, thus allowing the capsule to drop into the catalyst, solvent mixture. The agitation was started again and continued until absorption was complete as shown by a bonstant burette reading. The burette reading, temperature and barometric pressure were again noted and used to calculate the final volume of hydrogen.

Corrections to be applied to the readings for changes in temperature and barometric pressure depend on the free volume of the apparatus. The volume of the apparatus was measured by filling it to the top of the burette with water and measuring the amount of water it contained. Temperature changes produce variations in both partial pressure of solvent and volume of the gas. Corrections for both these factors as well as for temperature changes were determined by the following formula:

Total volume of H_2 corrected to standard conditions

$$(\text{volume of solvent})\left(\frac{237}{T}\ \frac{P-Vp}{760}\right)$$

where T = absolute temperature

P = barometric pressure

Vp = vapor pressure of ethyl alcohol at temperature T

The initial and final volumes of hydrogen were calculated with the aid of this formula. By subtraction, the volume of hydrogen absorbed by the sample was obtained. The hydrogen number (milligrams sample equivalent to one millimal of hydrogen) was then calculated by the formula, $H_2No.=\left(\frac{\text{mg sample}}{\text{cc hydrogen}}\right)(22.4)$. To calculate the volume of hydrogen absorbed by the sample in t terms of moles hydrogen per mole of methyl linoleate, the formula $\text{mol/mol}=\frac{(\text{cc hydrogen})\ (294.5)}{(22.4)\ (\text{mg sample})}$ was used.

Iodine Value. The method used for the iodine value was a modification of the Wijs method (16). A sample of approximately 100 milligrams was weighed into a 125 ml ground-glass

stoppered flask and dissolved in 5 ml of redistilled chloroform. Fifteen ml of Wijs' solution were added and the flask allowed to stand in the dark for one-half hour. Ten ml of 15 percent potassium iodine were added and the liberated iodine titrated with .0IN sodium thiosulfate solution using starch as an indicator. Blank determinations were made by the same procedure except no sample was present. The iodine number (grams iodine absorbed by 100 grams of sample) was calculated by the following formula:

$$\text{Iodine number} = \frac{(B-A) \times .01269 \times N Na_2S_2O_3 \times 100}{g \text{ (wt. sample)}}$$

The determinations were made in duplicate with a maximum allowable error of one percent.

Peroxide Value. The determination of the peroxide value of the oxidized material was carried out by a modification of the method of Wheeler (26). Approximately 100 milligrams of sample was weighed into a 125 ml Erlenmeyer flask and dissolved in 5 ml of a mixture of glacial acetic acid and chloroform (2:1 by volume). One ml of saturated potassium iodine solution was added and the mixture stirred by a rotary motion of the flask. Exactly one minute later 5 ml of water were added and the liberated iodine titrated with .0IN sodium thiosulfate solution. The end point was obtained by using starch solution as an indicator. It was necessary to shake the solution vigorously to remove the last traces of iodine from the chloroform solution. The peroxide content P,

expressed in millimoles of peroxide per kilogram of sample, is given by $P = \frac{T \times N \times 500}{W}$

Where T is ml of thiosulfate of normality N and W is weight of sample in grams. To convert this peroxide value to moles peroxide per mole of methyl linoleate the formula $\frac{P}{1000} \times .2944$ is used. The .2944 in the formula is molecular weight of methyl linoleate divided by 1000.

Determinations were made in duplicate with a maximum allowable error of one percent.

Spectropholometric Analysis. The ultraviolet absorption of the samples was determined by the use of a Beckmann D.U. Quartz Spectrophotometer. The sample, approximately 100 milligrams, was weighed directly into a 100 ml volumetric flask. The sample was dissolved in carefully purified absolute ethyl alcohol and the flask filled to the mark. Aliquot portions of this solution were then diluted with anhydrous alcohol until the reading at 2320 Å on the Beckman Scale ($\log 10 I_o/I$) was below 1.0. The scale readings were then calculated in terms of the specific absorption α by the method of Kraybill (13), ($\log 10 I_o/I)/C$ where C is concentration of sample in grams per liter. The percent conjugated material was calculated by the formula, specific α of sample /115 x 100. The 115 in this formula is the specific of pure Δ^{10-12} octadecadienoic acid.

Determination of Bromine. The bromine content of the

fatty acid derivatives was determined by the method described by Umhoefer (24).

A sample of 100 to 300 mg was weighed into a 125 ml Erlenmeyer flask provided with a ground glass stopper. Twenty-five ml of n-propyl alcohol and approximately 2 g of sodium cut into 4 or 5 pieces was then added. The flask was connected to a reflux condenser and refluxed for one to one and one-half hours. Excess sodium was decomposed by adding 15 ml of water, a few drops at a time through the condenser. The flask was taken from the condenser, phenolphthalein added and the solution neutralized with 6N nitric acid. Two ml excess acid was added. The bromine was precipitated with 3 percent silver nitrate solution, digested for ½ hour on a steam bath, filtered into a tared sintered glass crucible, dried at 100° and the crucible and silver bromide weighed. Percent bromine was calculated from the formula:

$$\%Br. = \frac{(.425)\ (Wt.\ AgBr)}{Sample} \times 100$$

Apparatus for Quantitative Oxidation of Methyl Linoleate

This apparatus consisted of a 200 ml reaction flask fitted with a ground glass neck. A short stopcock was sealed in the lower neck of the flask. The reaction flask was fitted with a ground glass connection and to this connection a short piece of glass tube was sealed. This tube was connected to a tube which led to the top of a 100 ml burette

by means of a short piece of thick walled rubber tubing. This allowed the reaction flask to be agitated by a motor driven eccentric while the rest of the apparatus remained stationary. Connected to the bottom of the burette was a T joint. One arm of the T was connected to a mercury filled leveling bulb and the other arm carried a stopcock. With this arrangement it was possible to sweep the whole apparatus with oxygen by lowering the mercury level in the burette below the stopcock, connecting the oxygen to the stopcock and opening both this stopcock and the one on the reaction flask. This was necessary as the apparatus was opened to obtain samples and the apparatus must be swept with oxygen and refilled with oxygen after the sample was obtained.

RESULTS

Bromine Substitution Reactions

The autoxidation reaction as postulated by Farmer and Sutton (9) involves a dehydrogenation of the active methylene group to form a free radical. Since some olefins will react with halogen to give the unsaturated halogen compounds, a similar dehydrogenation process must be involved. As the position of the substituted halogen is easier to locate than oxygen because of its characteristic reactions, a study of the action of bromine and hydrobromic acid on linoleic acid was undertaken to investigate the possibility of replacing hydrogen with bromine at the active methylene group.

Hydrobromic acid has an effect on the yield of crystalline tetrabromostearic acid produced when linoleic acid is brominated (14). This effect was thought to be a replacement of one hydrogen of the methylene group at C_{11} with bromine. Dry hydrogen bromide, produced by the action of bromine on wet phosphorous, was passed into a petroleum ether solution of linoleic acid at -70°. The hydrogen bromide liquified at this temperature. The reaction mixture was allowed to gradually warm to 0° with constant stirring. After all excess hydrogen bromide had bubbled out the solution was washed repeatedly with water, dried over sodium sulfate and the petroleum ether removed by vacuum distillation. The product obtained was a colorless oil, melting

point 0°-10°, it did not absorb iodine from Wijs' solution and contained 36.1 percent bromine (theoretical for dibromostearic acid 36.2 percent). According to these results, a mixture of the isomeric dibromostearic acids was obtained.

Another sample of linoleic acid was treated with hydrogen bromide as described previously until the iodine number dropped to 66.2 (theoretical for monobrom oleic acid 68.5). This material was a very viscous oil which would not solidify at temperatures down to -70° and contained 21.8 percent bromine. Attempts were made to hydrogenate the material but were unsuccessful. The material was brominated in Skelly solve F and absorbed 1 mole of bromine per mole to give a tribromostearic acid. On the basis of these data the material was identified as monobromo oleic acid. From these experiments it was evident that no substitution had taken place but only addition to the double bond.

Further attempts to prepare the 11-bromo linoleic acid were made. Seventy grams of methyl linoleate was dissolved in 500 ml of carbon tetrachloride, heated to reflux with two heat lamps and 43 g of bromine added slowly. Hydrogen bromide was evolved as the bromine was added. After all the bromine had been added, the solution was washed with water in a separatory funnel until the wash water contained no bromine. The solution was dried over sodium sulfate, and the carbon tetrachloride removed by vacuum distillation. The resulting material was a dark brown viscous oil with an

iodine number of 139 and contained 33.8 percent bromine. (Theoretical values of 11-bromo methyl linoleate, iodine number 135, percent bromine 22.1). Attempts to purify the material were made both by distillation and by crystallization. No pure monobromo methyl linoleate could be separated. Partial addition of the bromine to the double bonds as well as substitution had occurred.

The bromination of methyl linoleate with N-bromo succinimide was carried out. According to Zeigler, et al., (27), N-bromo succinimide contains a positive bromine atom that will replace a hydrogen atom at the allyl position in an olefin. The methylene group in linoleic acid between the double bonds is in the allyl position to both double bonds it should therefore be easily brominated with N-bromo succinimide. One-tenth mole of methyl linoleate was dissolved in 100 ml of carbon tetrachloride. One-tenth mole (13.9 g) of N-bromo succinimide was added to this solution and the mixture stirred for one hour at room temperature. The solid material was then filtered off and the solvent removed by vacuum distillation. A dark brown liquid remained which upon standing turned black. Attempts to crystallize the material met with no success. As the bromine was very easily removed, even with ice water, the material could not be washed free of impurities. Probably some 11-bromo methyl linoleate was formed but could not be obtained in a pure enough state to be useful.

Since all attempts to replace one hydrogen at C_{11} in

linoleic acid with bromine had failed this work was abandoned in favor of work with oxygen.

Effect of Conjugation on Iodine Numbers

The most convincing evidence for the theory of oxygen attack at the double bond of an olefin and subsequent formation of a cyclic peroxide has been the decrease in iodine value of the olefin as oxidation proceeds. Although there has been little correlation between the decrease in iodine value and peroxide content this has always been a point of argument between followers of the two theories of autoxidation.

That the iodine number of conjugated dienes is always below the theoretical value has long been known. The iodine number of a sample of Δ^{10-12} octadecadienoic acid was determined as 122.0, compared with the theoretical value of 181.4. The hydrogen value of the sample of Δ^{10-12} octadecadienoic acid, however, was theoretical (144.2 mg per millimole of H_2) thus showing that iodine numbers of materials that have a conjugated system of double bonds are not reliable. A shift of the double bonds to a conjugated system is postulated in an autoxidized sample of methyl, linoleate therefore the iodine values would be in error due to this conjugation. Hydrogen values will give theoretical results so the course of a typical autoxidation reaction of methyl linoleate was followed by the determination of peroxide,

hydrogen and iodine values. Tank oxygen was introduced into a sample of methyl linoleate at room temperature thru a sintered glass filter stick and samples of the oxidized ester removed at intervals for analysis. The oxidation was continued until the peroxide value had reached a maximum and started to decrease. The results of this experiment are shown in Fig. 2. The hydrogen, iodine and peroxide values were calculated in moles per mole of methyl linoleate and plotted against time of oxidation. The hydrogen values are shown both as determined and corrected for peroxide values. The results of this experiment show that the iodine values of the partially autoxidized samples are in error. The hydrogen values, however, are considered to give a true measure of the unsaturation if the values are corrected by subtracting the peroxide value of the sample from the hydrogen value.

Quantitative Oxidation of Methyl Linoleate with Molecular Oxygen

Quantitative oxidation of methyl linoleate with molecular oxygen was carried out in the apparatus described under experimental.

Forty-five ml of methyl linoleate was poured into the weighed reaction flask and the weight of sample determined. The reaction flask was connected to the burette and the system swept with oxygen for several minutes with the stopcock in the reaction flask open. The system was closed

and allowed to stand for a few minutes. The oxygen volume was adjusted to the bottom level of the burette by opening momentarily the stopcock in the flask and the temperature and barometric pressure determined. Agitation of the sample was then started and continued for several hours. Immediately before removing a sample, the volume, temperature and barometric pressure were determined. Because it is necessary to know the free volume of the apparatus to calculate the total oxygen present before and after absorption, the volume of sample withdrawn was measured by means of a pipette. To remove samples the reaction flask was disconnected from the measuring burette, 1 ml of sample removed, weighed and used for determination of peroxide oxygen, hydrogen absorption, and spectrophotometric analysis. After removal of the sample the reaction flask was connected to the burette, the apparatus swept out with oxygen and adjusted as before, and agitation continued. The volume of oxygen used by the methyl linoleate present in the reaction flask was calculated by subtracting the total volume of oxygen at standard conditions present after absorption from the total volume at standard conditions before absorption. The reaction was carried out at room temperature which was $27^{\circ} \pm 2$.

The results are given in Fig. 3 calculated in moles per mole of methyl linoleate. Since the volume of oxygen absorbed was measured between samples, the moles oxygen absorbed by the moles of methyl linoleate present in the

reaction flask was calculated for each sample and added to the total moles absorbed previously. As shown in Table 1, all oxygen absorbed could be demonstrated as peroxide oxygen up to a peroxide concentration of .2 moles per mole of methyl linoleate. After this peroxide concentration is reached there is an increasing difference between the peroxide oxygen and total oxygen absorbed. The hydrogen values show the decrease in unsaturation to be very small until excess oxygen appeared. At this point the decrease in unsaturation assumes a more rapid rate. The percentage of conjugated material in the oxidized samples show an increase until shortly before the peroxide peak is reached and then starts to decrease. If it is assumed that only peroxidized molecules are conjugated, the data show approximately 75 percent of the oxidized molecules are conjugated. The percentage of conjugated material decreases after excess oxygen appears and at almost the same point, a slight increase in the gain in specific gravity is noted. The decrease in unsaturation is greater than the decrease calculated by assuming that 1 mole of excess oxygen saturates one double bond. This is true until one mole of total oxygen has been absorbed. At this point one half the oxygen absorbed is peroxide oxygen and the observed decrease in unsaturation corresponds with the calculated decrease of one half mole. After one mole of oxygen has been absorbed, the calculated decrease is greater than the observed decrease in unsaturation.

Polymeric Tendency of Conjugated Linoleic Acid

Oxidation of methyl linoleate causes a shift of the isolated system of double bonds to a conjugated system. The polymeric tendency in conjugated systems is greater than isolated systems. To show this, a sample of pure Δ^{10-12} octadecadienoic acid was oxidized at 88°C by bubbling tank oxygen into the sample. The high temperature was used because of the high melting point of the acid (52.5°). To furnish a comparison, a sample of linoeic acid was oxidized under the same conditions. The results are given in Fig. 4. It was shown that the double bonds in conjugated linoleic acid are destroyed at a much faster rate than in the normal linoleic acids. Peroxide formation was very limited in the conjugated material. The peroxide peak of the normal linoleic acid was very low showing that the peroxide breakdown is accelerated by heat.

After the hydrogen absorption determinations had been made, the alcohol solution was extracted with Skelly solve F, the solution dried over sodium sulfate and the solvent removed under vacuum. In all partially oxidized samples, the hydrogenated material consisted of stearic acid crystals suspended in a gummy material. The amount of crystals present depended on the extent of oxidation of the sample, less being present in samples that were oxidized longer. This indicates that polymerization of the acids was taking place while being oxidized, with the conjugated acid being polymerized at a more rapid rate than the nonconjugated acid.

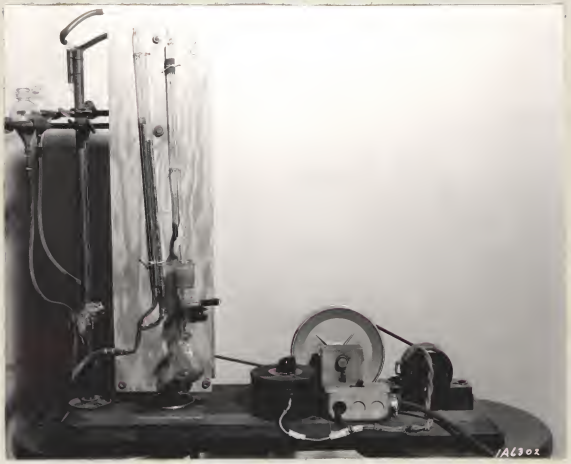


Fig. 1. Apparatus for determination of hydrogen values.

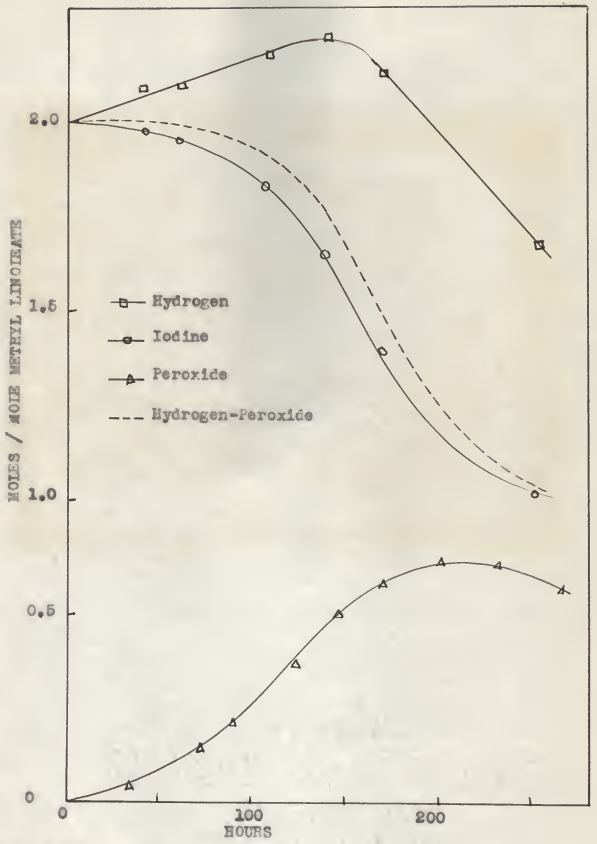


Fig. 2. The oxidation of methyl linoleate showing error in iodine values.

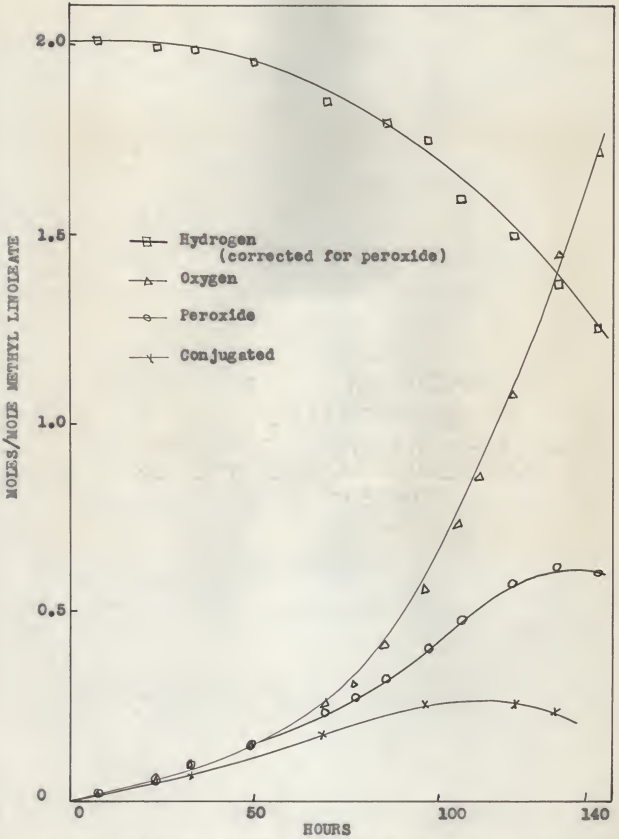


Fig. 3. The quantitative oxidation of methyl linoleate.

Table 1. The quantitative oxidation of methyl linoleate.[1]

Time	Peroxide	Oxygen Observed	Oxygen -Peroxide	Increase in Saturation (2-hydrogen)	Conjugated (% of oxidized)	Specific Gravity
0	0	0	0	0	.8	.880
7	.031	.030	0	0		.889
23	.075	.074	0	.01		.903
33	.100	.096	0	.01	76	.909
49	.141	.141	0	.05		.911
69	.240	.255	.015	.14	71	.918
77	.273	.312	.049	.15		.927
85	.324	.411	.097	.22	81	.931
96	.402	.556	.154	.25	65	.941
105	.478	.736	.258	.40	52	.941
119	.572	1.075	.503	.576	42	.961
131	.608	1.445	.873	.620		1.01
142	.600	1.701	1.101	.754		1.03

[1] All values given as moles per mole methyl linoleate

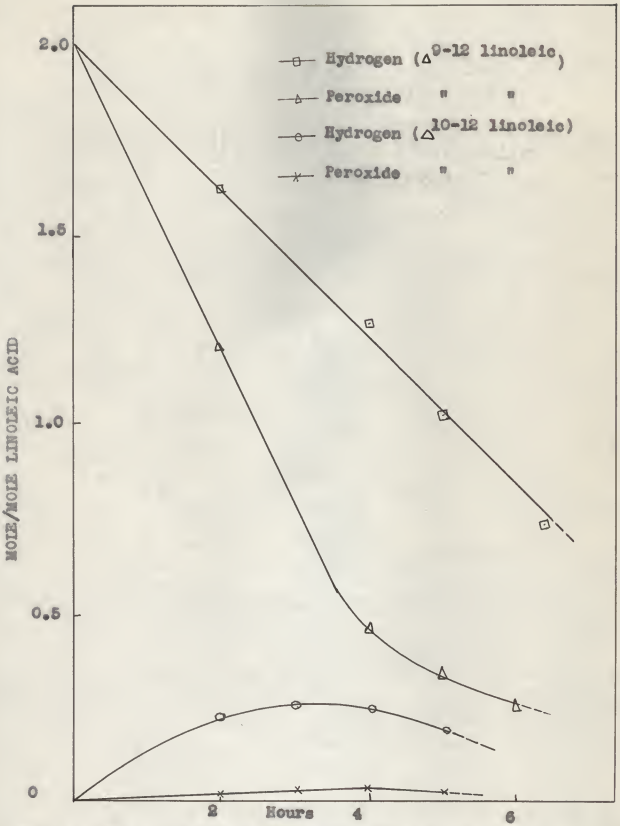


Fig. 4. The oxidation of conjugated and non-conjugated linoleic acids.

DISCUSSION

The data presented in this paper add evidence to the theory that hydroperoxides are the initial product formed when methyl linolate is autoxidized.

Hydrogen has not been used by previous workers to measure unsaturation in partially oxidized materials. Iodine chloride or iodine bromide solutions were used previously but the use of these reagents to measure unsaturation was shown to give erroneous results when applied to conjugated systems of double bonds. It is believed that this error is caused by 1,4- addition of the halogen to the system -CH:CH-CH:CH-. (15). The halogen adds to the 1,4 positions rapidly with formation of a new double bond at the 2,3 position. Halogen will add very slowly if at all to this double bond thus causing errors in the iodine values. Since the double bonds in autoxidized methyl linoleate were shown to be conjugated, hydrogen was believed to give results which were more exact if a correction for the hydroperoxide was made on a mole per mole basis. Hydrogen values, however, might be in error due to the reduction of aldehydes and ketones which are believed to be produced by secondary reactions. However, the amount of aldehydes and ketones was considered to be small and the error caused by their presence to be within the experimental errors of the determination.

The data supported the theory that dehydrogenation of the active methylene group with a consequent shift of double

bonds to a conjugated system occurred. The oxygen uptake of methyl linoleate shows an autocatalytic curve that would be typical of a free radical mechanism as described by Bolland and Koch (3). The initial reaction product shows about 80 percent conjugation which is higher than would be expected if all three mesomeric forms of the free radical show the same reactivity toward oxygen. Bergstrom's (2) failure to isolate the 11 hydroxy stearic acid after hydrogenating partially oxidized material would indicate that the two mesomeric forms which contain conjugated double bonds were more reactive than the form which contains an isolated system. The ultra violet absorption data could be in error due to the great variety of secondary products present in the autoxidized ester, therefore the spectrophotometric data must be taken with reservations, especially after secondary reactions have started.

The hypothesis that oxygen is attached to the carbon chain at some other point than the double bond is supported by the data. In all oxidations conducted at room temperature, the hydrogen absorbed by the initial product of oxidation was over the two moles necessary to saturate the double bonds present. Since a hydroperoxide group is easily reduced to a hydroxyl group and water by one mole of hydrogen, this excess hydrogen was used in the reduction of the hydroperoxide group.

Chemical evidence as to the mechanism of breakdown of

the primary hydroperoxides of olefins is much more obscure than that relating to their formation. That the hydroperoxides do break down was shown by the data obtained by measuring the total oxygen absorbed and peroxide oxygen present. It was found that after about 0.2 mole of oxygen was absorbed, non-peroxide oxygen was present in a constantly increasing amount as oxidation proceeded. This excess oxygen could have been in two forms. One, oxygen that had been attached to the molecule as hydroperoxide and then decomposed, or, two, oxygen which had reacted initially with the methyl linoleate to give some other type of group than a hydroperoxide. Since in the very first stage of the reaction all the oxygen is present as peroxide oxygen, there would be no reason to assume that after oxidation proceeds to a certain point, a different initial product is former. Therefore, all oxygen that cannot be demonstrated as peroxide oxygen must have gone through the hydroperoxide stage and then decomposed to give hydroxyl groups and activated oxygen. This active oxygen is capable of reacting with the double bonds to give various compounds such as epoxy, hydroxyl, ketonic, aldehydic and ether linkages intermolecularly to form dimers. The peroxide decay also has the typical curve of an autocatalytic reaction. This would explain the peroxide peak that is noted. Peroxide formation has been overtaken by peroxide decay at the peroxide peak. As the peroxide value falls, the peroxide decomposition is proceeding faster than peroxide formation.

The reaction of the activated oxygen that is produced by hydroperoxide decomposition seems to react preferentially with the conjugated system as shown by the decrease in percentage of conjugated dienes as the oxidation proceeds. A different reaction was shown to take place when conjugated linoleic acid was oxidized. The peroxide formation was practically zero and saturation of the molecule proceeded at a much faster rate than when the unconjugated acid was oxidized. The reaction of molecular oxygen with a conjugated diene appears to be an initial attack at the double bond without peroxide formation.

The variety of products obtained from the secondary reactions of hydroperoxides make any quantitative study of the reaction very difficult. Temperature and method of introducing oxygen seem to influence the reaction. The hydroperoxide groups are decomposed thermally and an increase in temperature will cause an increased rate of decomposition of peroxide. The initial reaction or hydroperoxide production rate is also increased but not as much as is peroxide decomposition. Autoxidation studies at room temperature give the best results. The hydroperoxide is stable enough at room temperature that considerable hydroperoxide is built up in the system before decomposition starts.

SUMMARY

Iodine values by the Wijs' method were shown to be in error when compared to hydrogen values as a measure of unsaturation in partially autoxidized methyl linoleate.

The total oxygen absorbed initially by methyl linoleate was shown to be present as a hydroperoxide with both double bonds intact but in later stages of oxidation oxygen was present in the molecule which could not be demonstrated as peroxide oxygen. This oxygen was presumed to arise from hydroperoxide decomposition and subsequently to react with the double bonds present to form various products.

Conjugated (Δ^{10-12}) linoleic acid was shown to react in a different manner with molecular oxygen than the normal (Δ^{9-12}) linoleic acid.

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