DIFFERENCES IN THE AUTOXIDATION OF LINOLEIC ACID AND ALKALI CONJUGATED LINOLEIC ACID

by

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B. S., Kansas State College
of Agriculture and Applied Science, 1947

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1948

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INTRODUCTION

The process by which fats and fatty acids undergo spontaneous oxidation when exposed to the atmosphere is of great significance from both a biological and economic standpoint. This action of air on fats and other unsaturated materials may be advantageous or undesirable depending on the conditions and circumstances under which such action takes place. Thus "blown oils" are produced and paints, varnishes and lacquers carry out their function. On the other hand, protective films deteriorate because of excessive oxidation. Moreover, fuel and lubrication oils develop sludges; rubber becomes brittle; food gets rancid; peroxidized fuels cause corrosion in internal combustion engines; and essential oils resinify when acted upon by molecular oxygen. It follows that this type of oxidation, which has been termed autoxidation in view of its spontaneous nature, is for the most part undesirable as it so often causes serious economic losses.

Autoxidation is characterized by a long "induction period" during which oxidative action occurs at a very slow and nearly constant rate, followed by a period of rapid reaction rate which increases logarithmically up to a limiting value and then declines. An autoxidation is sensitive to the presence of small amounts of foreign substances. Thus, some substances promote the oxidation by shortening the induction period, whereas other

substances lengthen this period. These substances are called pro-oxidants and antioxidants, respectively. It has been established experimentally that neither antioxidants or pro-oxidants have any outstanding effect upon the oxidative rate achieved during the phase of rapid reaction. They merely hasten or delay the beginning of that phase.

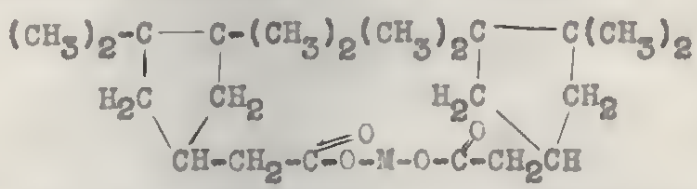
Typical Pro-oxidants

The way in which pro-oxidants shorten the induction period allowing the rapid phase of the reaction to start sooner is not well understood. Nevertheless, two general modes of action have been suggested: (1) the pro-oxidant may actually catalyze the union of oxygen with unsaturated fats; (2) the pro-oxidant simply serves to destroy any other substances or entities which would otherwise inhibit the oxidation.

Many metals and a few organic substances exhibit a pro-oxidant effect. In the case of metallic driers the order of decreasing activity may be represented as follows (12): Co>Mn>>Cu>Ce>Pb>Cr>Fe>U>Bi>Ca>Ag>Zn>Th>Hg>Sn>Ni>Al . In regard to organic substances, carotene, benzoyl peroxide and certain oxidation products of ascorbic acid show pro-oxidant activity. In addition, ultraviolet light seems to favor autoxidation.

At present, the metallic salts of naphthenic acids are recognized as the most ideal driers. These cycloparaffin derivatives (39) have the general formula $C_nH_{2n-1}COOH$. They are

found in crude petroleum and have boiling points ranging from 216 C. for hexahydrobenzoic acid to 310 C. for the C_{14} acid. A typical drier may be represented as follows (16):



where M represents a metal; lead, manganese and cobalt being the most widely used. An excellent discussion of the preparation of naphthenate driers has been presented by Greenfeld (39). Curwen (16) has demonstrated that the naphthenates are generally superior to the resinates, linoleates and oxides as commercial paint driers.

Although the qualities, uses, and action of driers have been discussed (16, 23, 36, 39), the mechanism by which they work has not been clearly or completely defined. A number of workers have classified driers as psuedo rather than true catalysts. Riemenschneider (53) states that driers increase the efficiency of energy transfer from one molecule to another. Elm (22) speaks of the dissolved metal oscillating between two stages of oxidation, thereby allowing it to activate the oxygen of the air and pass it on to the drying oil molecules. Still other workers express the view that the drier promotes the formation of, or stabilizes the peroxide.

It is now generally believed that a drier not only accelerates the rate but also modifies the course of the oxidation because the total amount of oxygen absorption during paint

drying is less when a drier is present. Furthermore, the amount of carbon dioxide is the same with or without drier.

By dividing the drying into three steps; viz., oxidation, polymerization, and coagulation, paint and varnish chemists have come to the following conclusions as outlined by Elm (22):

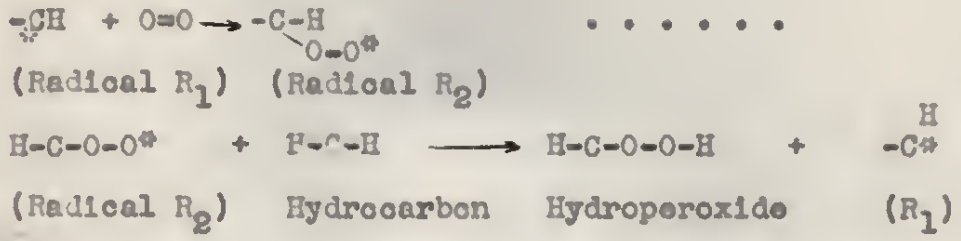
> 1. The driers are primarily accelerators of the oxidation reaction, neutralizing antioxidants and increasing the oxidative rate.
>
> 2. The driers accelerate association or polymerization of the oxidized oil molecules by increasing the concentration of polar molecules and exerting an orientating influence upon them.
>
> 3. The driers accelerate gelatin by serving as coagulation centers or nuclei.
>
> 4. To fulfill such a triple role the drier must be in an available form and occur in two stages of oxidation of which the higher one is stable in air but unstable in a drying oil, and the lower one is stable in oil but unstable in air.

The very excellent functional mechanism laid down here has served well, but its chemical counterpart in drying oils remains to be postulated. The investigation in this laboratory was concerned with the chemical action of the drier in the autocatalytic phase; viz., what is ordinarily the first few hours of drying time.

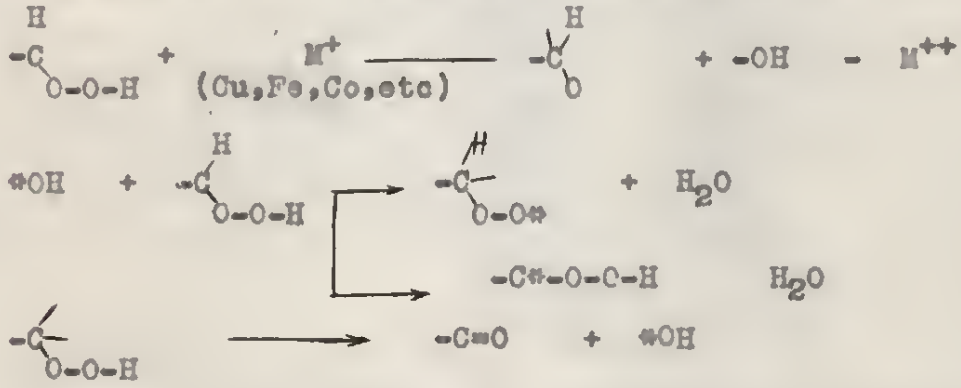
It is fair to say that a chemical mechanism for drier action during the autoxidation of tetralin has been postulated. Yamada (61) found that heat increases the decomposition velocities of tetralin and turpentine peroxides and that these decomposition reactions were first and second order, respectively. In addition, he proved that Mn, Co, and Pb compounds raised the

reaction order one step. Cook (13) and Ivanov et al.(43) showed that the transition metals promoted the decomposition of tetralin hydroperoxide.

In the summer of 1945, Robertson and Waters (54) wrote assuming the 1-methylene group of tetralin oxidized, as follows:



that the role of the metal may be postulated thus:



It is evident then that these metallic catalysts by promoting the hydroperoxide decomposition increase the concentration of the free *OH radical.

Robertson and Waters further state that these secondary catalysts become more active after the oxidation has proceeded for a short period and that an electronic balance is set up between organic peroxides and metallic cations to regenerate the

latter:

$$M^{++} + R\text{-}O\text{-}O\text{-}H \longrightarrow M^{+++} + R\text{-}O\text{:}^{\ominus} + {}^{\bullet}OH$$

$$M^{+++} + R\text{-}O\text{-}O\text{:}^{\ominus} \longrightarrow R\text{-}O\text{-}O^{\bullet} + M^{++}$$

Further, that once the equilibrium between the oxidized and reduced states of the metallic cation has been reached the relative concentrations of the active organic radicals also will become steady. This explains why it is of no particular advantage to increase the concentration of metallic autoxidation catalysts above a certain value.

It is doubtful that this mechanism is identical with the one followed by driers in more unsaturated systems in view of the increased complexity and greater quantity of free radicals formed in the latter. It is safe to say, however, that the decomposition promoting role of these catalysts is probably duplicated when olefins are autoxidized in their presence. Just how the bound metal ($-\overset{O}{\overset{\|}{C}}\text{-}O\text{-}M\text{-}O\text{-}\overset{O}{\overset{\|}{C}}$) might reach the ionic state in an organic system is open to question.

Antioxygenic Activity

In its action, an antioxidant works against the processes by which hydroperoxides are built up and unlike a pro-oxidant, actually undergoes oxidation. Following the destruction of inhibitor, the autoxidation then proceeds at a rate which is independent of the previous existence of the antioxidant.

In regard to structure, the phenolic nature of this group

of compounds is recognized as general. Moreover, most of them have electronic configurations similar to ortho and para diphenols though non-phenolic antioxidants have been suggested; e.g., thio ether, thiourea, phospholipids and a number of the amino acids (53).

Whatever the type of antioxidant, the precise manner in which they work has in no case been determined. Backstrom et al. (3, 1) assumed that autoxidation utilizes a series of chain reactions (5, 11) and represented inhibitor molecules acting as efficient chain terminators undergoing complete oxidation in the process. Riemenschneider (53) recently wrote that the first step in oxidation involved the union of a molecule of oxygen with one of fat. The peroxide formed transfers its energy to another fat molecule which in turn reacts with oxygen. In this way a series of chain reactions are initiated which continue to completion unless otherwise halted. The oxidative steps may be represented as follows (53):

1. $R + \text{energy} \rightarrow R^*$; $O_2 - \text{energy} \rightarrow O^*_2$
2. $R^* + O_2 \longrightarrow RO^*_2$; $R + O^*_2 \longrightarrow RO^*_2$
3. $RO^* + R \longrightarrow RO_2 + R^*$
4. $R^* + O_2 \longrightarrow RO^*_2$ etc.

where R = fat, RO_2 = peroxide and the asterisk indicates free radical nature.

An antioxidant (A) may inhibit the reaction chains by one or more of the following methods:

5. $A + R^{*} \longrightarrow A^{*} + R$; $A^{*} + O_2 \longrightarrow AO_2$

6. $A + O_2^{*} \longrightarrow AO_2$

7. $A + RO_2^{*} \longrightarrow A^{*} + RO_2$

8. $A^{*} + RO_2^{*} \longrightarrow AO^{*} + RO^{*} \longrightarrow A + R + O_2$

9. $AO^{*} + R \longrightarrow A - RO^{*}$

Golumbic (37) using alpha-tocopherol and Filer et al. (33) gallic acid, found experimental evidence which supported the sacrificial role of (A) as represented in steps 5 to 7. The regenerative role as indicated by 8 and 9 has also been supported by the work of Golumbic (38).

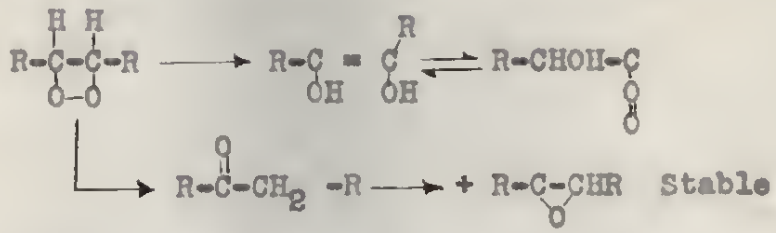
To date this is the best mechanism proposed and to many serves as a completely reasonable explanation of inhibition.

Theories of Autoxidation

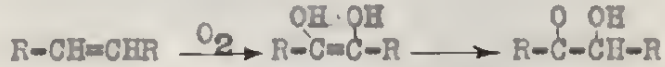
Every theory attempting to explain the process of autoxidation has been based upon some mechanism involving the initial addition of oxygen to the reacting molecule and upon the product of this addition. Since Shonbeim, according to Markley (47), first investigated the effect of oxidizing agents on almond oil and turpentine, numerous qualitative and quantitative studies of autoxidation have been made. The modern theories arising from these studies date from the work of Engler and co-workers (23-25) who stated that autoxidation by atmospheric oxygen is molecular rather than atomic in character.

In 1909 Fahrion (26) stated that an autoxidation of fatty

acids produced a cyclic peroxide which underwent rearrangement as follows:



Ellis (17-20) believed that molecular oxygen added to the double bond followed by partial rearrangement:

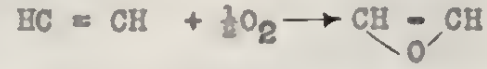


Staudinger (55) proposed that molecular oxygen added to the double bond to form an unstable moloxide which rearranged to a peroxide compound:

moloxide

It is interesting to note that none of the initial products represented above has ever been isolated.

Fokin (34, 35) proposed that the initial product of autoxidation was an ethylene oxide ring now called an epoxide:



Fokin collected considerable kinetic data to support this reaction and the fact that numerous epoxides and oxido acids have since been isolated has caused its incorporation with modifications into the hydroperoxide theory.

Following the suggestion of Criegee (14, 15) that cyclo-

hexene autoxidized to form a hydroperoxide which had the structure  Rieche (52) suggested that unsaturated fats and oils reacted in a similar fashion due to the presence of active methylene groups:

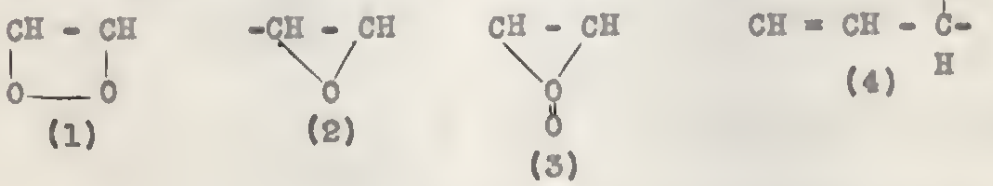
$$-C{=}CH + CH_2 - CH{=}CH \xrightarrow{O_2} -CH{=}CH - \underset{H}{\overset{OOH}{C}} - CH - CH = CH-$$

or

$$-CH{=}CH - CH_2 - \overset{OH}{CH} \xrightarrow{O_2} -CH{=}CH - \overset{OOH}{\underset{H}{CH}} - \overset{OH}{\underset{H}{C}} - H$$

The oxidation of cyclohexene (57) and tetralin (41) have greatly substantiated Rieche's suggestion.

At this point there exists four suggested initial products: (1) a cyclic peroxide, (2) an epoxide, (3) a moloxide and (4) a hydroperoxide. Their structures follow:

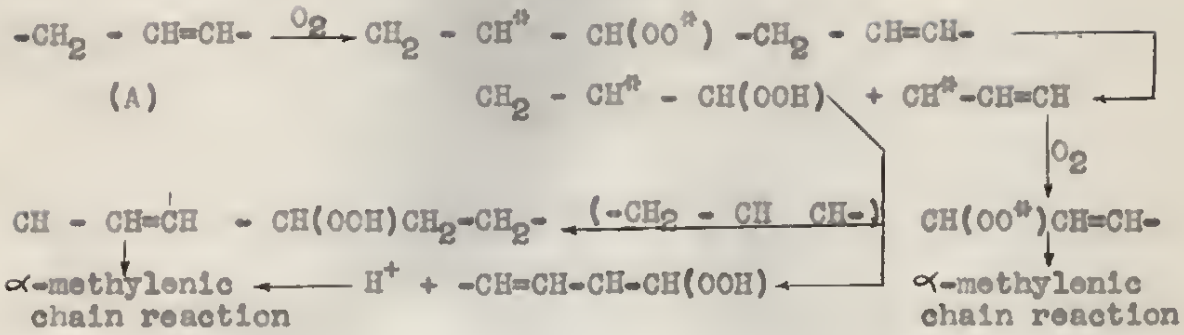


Let it suffice to say that (1) and (3) have not received sufficient experimental support to render them acceptable. On the other hand, (2) has been incorporated into the hydroperoxide theory whose primary initial product is represented in (4).

The alpha-carbon atom in unconjugated olefins is the one attached by the molecule of oxygen to form a hydroperoxide having an intact double bond.

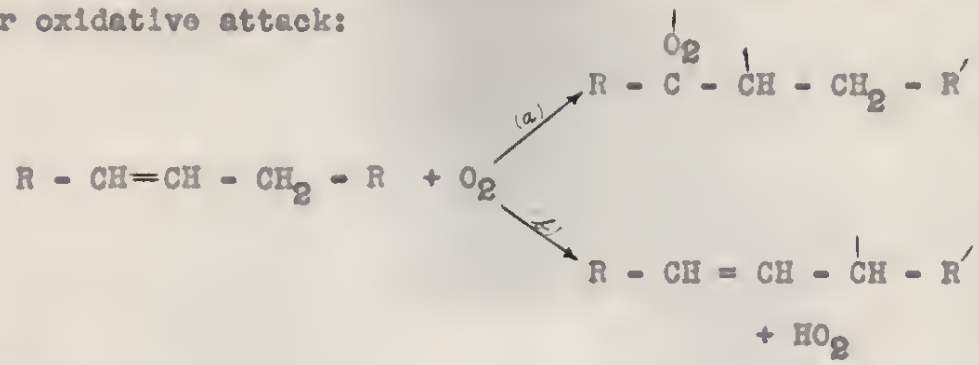
Farmer (27, 32), Farmer et al. (28, 29, 30, 31), Walsh (59) and Bolland et al. (6-10) hold the view that olefinic peroxidation occurs by way of a free radical mechanism which

Farmer assumed to pass through the stages:



in which the free radical center is indicated by an asterisk. Then, as represented, it is the alpha-methylenic chains which carry the reaction to completion but it is the momentary addition of oxygen to a few of the double bonds present in (A) that initiates these chains. By way of further explanation. Farmer stated that the extent of actual initial addition at the double bonds is relatively insignificant but does take place and that an intramolecular reaction of a type permitting this first formed diradical $-CH_2 - C^*H - CH(OO^*)$ to become stabilized by detachment of hydrogen from the adjacent - methylene group (⟶ -CH=CH - CH - (OOH) is unlikely since it would not propagate chain reactions. This initial step of momentary addition to the double bond was suggested in order to conform with the work of Bolland and co-workers (7, 8) who estimated that it would require 80 K calories of energy to detach a hydrogen atom from an alpha-carbon in an olefine system and 64 K calories to convert - C=C- to -C -C -. It is fair to say that the source of the necessary initiating energy (≧64 K cal.) remains a mystery.

Later Bolland and Gee (9) recognized two possible sites for oxidative attack:



Where

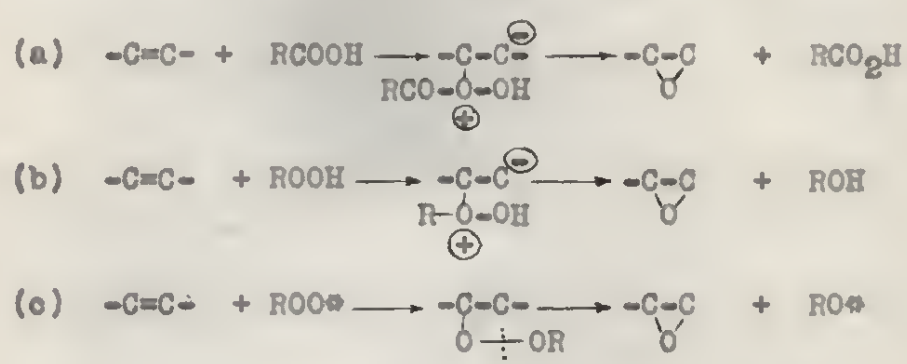
H_a = 14 K cal.

H_b = 7 K cal. (these heats were estimated)

According to these workers double bond attack in a 1:4 diolefin may be equivalent to that of a monoolefin as represented above, but alpha-methylenic attack will be easier by virtue of 11 K cal. greater resonance energy in the radical. Neither of these suggested initial reactions can be ruled out since their heats lie below the energies of activation found experimentally. The absolute rates of neither (a) nor (b) have been determined because the smallness of these rates renders them difficult to observe. Moreover, even a small amount of impurity would exert a relatively large influence on the site of initial attack. A mechanism has been postulated by Farmer et al. (31) stating that peroxide decay occurs, side by side with new peroxidation, releasing active oxygen which is used in oxidizing either the adjacent or some remote double bond, the latter perhaps in another molecule.

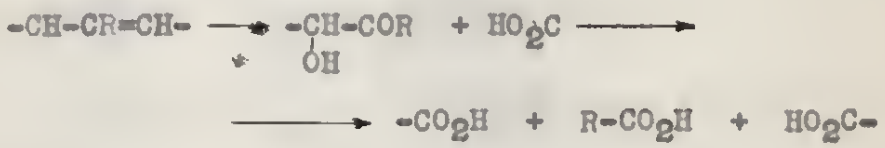
The basic reactions involving the alpha-methylenic group were represented as follows:

1. Reaction between double bonds and peroxide group

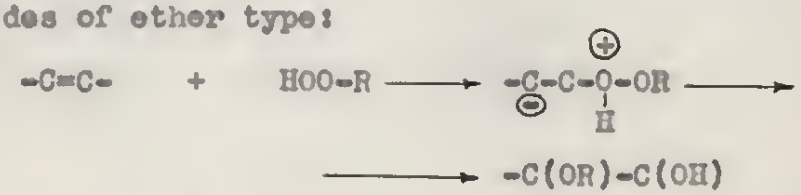


Reaction (c) is intermolecular; the peroxide group in one molecule reacts with a double bond in another molecule or a remote double in the same molecule.

2. Secondary splitting between a double bond and an alpha-carbon atom

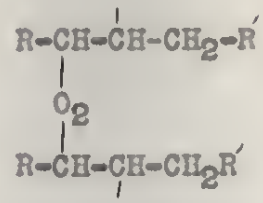


3. Polymerization by interaction of -OOH and double bonds to give dimerides of ether type:

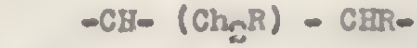


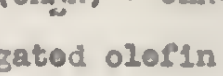
In addition RO* and ROO* in step (1) may react with other olefinic systems present.

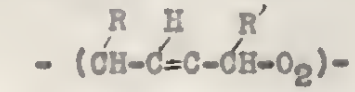
Bolland and Gee (9) represented the dimer for a mono-olefine as



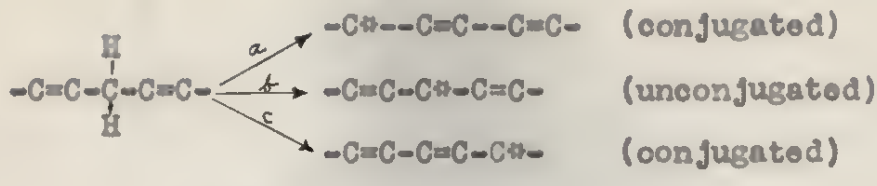
in which the free radical ends could react with oxygen producing further peroxide radicals which could again add olefin. In this way, a polymeric chain would be built up with the repeating unit:

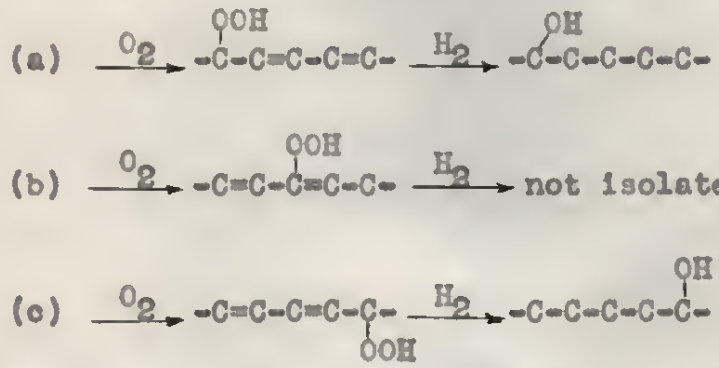


In the case of a conjugated olefin () the terminal carbons could react with oxygen giving the polymeric chain



The steps 1, 2, and 3 were suggested after Farmer and Sutton (29) isolated for the first time a fairly pure peroxidized methyl oleate which they found to be a mixture of two isomeric monohydroperoxides each containing a molecule of oxygen and one double bond. Quite independently Bergstrom (4) isolated 9 and 13 hydroxystearic acids by chromatographing the products obtained from the hydrogenation of autoxidized linoleic acid. He concluded that oxygen attacks at the alpha-methylene group producing a free radical which resonates between three equivalent structures. Although Bergstrom failed to isolate the 11-hydroxystearic acid, the 9 and 13 acids may be explained with resonance forms as follows:





In support of the above representation, Hilditch and Gunstone (40) studied the autoxidation of ethyl linoleate at various temperatures and found that the development of diene conjugation paralleled the formation of peroxides. Moreover, Bergstrom found that linoleic acid oxidized at 37° C. contained peroxides which did not exhibit diene conjugation and which on hydrogenation yielded alpha-glycolic groups to the extent of 0.2 mole for every mole of oxygen absorbed.

To summarize, there are now two schools of thought concerning the autoxidation of olefines. Both, however, agree that the alpha-methylene group is intimately involved. On the one hand, Bergstrom and others (9) believe that oxygen initially adds directly onto the alpha-methylene group whereas Farmer (27, 32) and Farmer et al. (28-31) suggest that molecular oxygen adds to a few of the double bonds momentarily and then forms hydroperoxides at the methylene group. Just how the hydroperoxides are

formed is still open to question, a concise formulation necessarily resting upon the exact determination of the thermochemical quantities involved and upon probability calculations.

Interest in the factors which affected stability of highly unsaturated fatty acid (44, 45) and in the oxidative mechanism prompted this study of the autoxidation of linoleic acid.

The rates of autoxidation of conjugated and nonconjugated acid were compared and the modifications brought about by the addition naphthenate driers[1] and phenolic inhibitors noted.

EXPERIMENTAL

Preparation of Materials

Unless otherwise indicated, the 9, 12 linoleic acid used in this investigation was obtained via the ethyl ester which was prepared by the debromination of tetrabromo stearic acid by the Rollett (55) method as modified in this laboratory. The conjugated acid prepared was of two varieties. The liquid isomer was made up of 10-12; 9-11; and 9-12 acid whereas the solid acid was composed of the 10-12 isomer entirely. The former was prepared by a modified method of Holman and Elmer (42), the latter by a modified method of Van Mikusch (58). Efforts were made to obtain a very pure product. The iodine,

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[1] Naphthenate driers, obtained through the courtesy of S. B. Elliott, Ferro Chemical Corporation, Cleveland 14, Ohio.

peroxide and ultraviolet absorption, values served as criteria for this purity.

The techniques used for the preparation and utilization of these and other compounds are described in some detail in the following pages.

Preparation of the Fatty Acids. Three hundred g of potassium hydroxide were placed in a five liter round bottom flask. Solution was effected with 100 ml of water and 1500 ml of ethyl alcohol. The solution was heated to boiling on a steam bath, 1000 g of corn oil[2] added and the mixture refluxed for one hour. The resulting hydrolysate was cooled to room temperature in a stream of tap water and 2000 g of cracked ice in 1000 ml of distilled water added. This soap solution was neutralized by adding 575 ml of concentrated hydrochloric acid in small portions with constant stirring. The cold acidic mixture was placed in a five liter separatory funnel and extracted by adding one liter of Skelly B in 250 ml portions. The Skelly layer was washed with water and dried in a three liter round bottom flask with 100 g of anhydrous sodium sulfate. The dry solution was diluted with 1000 ml of redistilled Skelly B and chilled overnight at -10° C. The saturated acids and sodium sulfate were filtered off and 1000 ml of Skelly B added to the filtrate which was then ready for bromination.

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[2] A degummed corn oil furnished through the courtesy of Corn Products Refining Company, Argo, Illinois.

Preparation of Crystalline Tetrabromostearic Acid. The five liter flask containing the solution of fatty acids was clamped securely into an ice-salt bath. A mechanical stirrer was adjusted to about one inch from the bottom of the flask and bromine added to the cold solution at a rate such that the temperature of the reaction mixture did not rise above 10° C. When optimum saturation had been reached, as evidenced by a faint pink color, the flask was corked tightly and chilled at -10° C. overnight. The resulting crude crystalline tetrabromostearic acid was collected on a 25 cm Buchner funnel. These crystals were repeatedly recrystallized from a three to one solution of Skelly B and diethyl ether to a constant melting point of 115° C.

Preparation of Ethyl Linoleate. Two hundred g of pure tetrabromostearic acid and 200 g of clean, granular zinc were mixed together and placed in a dry, ground-glass, five liter, round bottom flask. A 60 cm condenser was attached, 400 ml of absolute alcohol added, steam heat applied for two minutes, the steam turned off, and the exothermic reaction allowed to proceed. When the initial reaction had subsided, the mixture was refluxed for two hours. Then 0.4 ml of concentrated hydrochloric acid was added and the reflux resumed for 30 minutes. The reaction mixture was decanted from the zinc, chilled with 200 ml distilled water and extracted with Skelly B. The aqueous phase was then discarded and the Skelly solution of the ester washed with distilled water.

Preparation of 9-12 Linoleic Acid. One hundred g of the ester was placed in a three liter round bottom flask and saponified with 500 ml of 10 percent alcoholic potash by standing overnight at room temperature. An equal volume of distilled water was added and any unsaponified ester extracted with Skelly F. Cracked ice was added to the soap solution which was acidified with dilute hydrochloric acid with gentle shaking. The mixture was placed in a four liter separatory funnel and extracted with 1500 ml of Skelly F. The resulting solution was washed with water and dried over 50 g of sodium sulfate. The solvent was removed under vacuum. The pale yellow liquid residue was distilled at a reduced pressure of 100 mu and 150° C. with a Glasco heater and a variable voltage transformer. The colorless distillate had an iodine value of 180, a peroxide number of zero and a specific absorption value of 0.324 at 2320 Å.

Preparation of Liquid Conjugated Acid. To 600 ml of ethylene glycol containing 100 g of potassium hydroxide at 180° C., 50 g of 9-12 linoleic acid was added dropwise with stirring. A tank nitrogen atmosphere was maintained. The temperature was held at 175°-180° C. for 30 minutes when the mixture was poured into four volumes of cracked ice. The soaps were neutralized with cold concentrated hydrochloric acid and extracted with Skelly F. This Skelly solution was washed with water and dried over sodium sulfate. The conjugated acid remained as a pale yellow residue upon the removal of solvent

with a water pump. The last traces of solvent were removed with an oil pump. The acid possessed an absorption value of 85.5 at 2320 Å.

Preparation of Methyl Linoleate. Two liters of redistilled methyl alcohol were placed in a five liter round bottom flask and 1000 g of dehydrated castor oil[3] added. Then 50 ml of concentrated hydrochloric acid were added and the mixture refluxed on a steam cone for 60 hours. One liter of distilled water was added and the mixture extracted in a five liter separatory funnel with 2000 ml Skelly F. The solution was dried over sodium sulfate and the solvent removed from the ester under vacuum. The impure methyl ester was distilled at 110° C.mu and 140° C. to give a colorless distillate having an absorption value of 34.6. Yield 650 g.

Preparation of the Crystalline Conjugated Acid. To 1430 ml of ethylene glycol containing 520 g potassium hydroxide at 180° C., 200 g of the methyl ester were added dropwise with constant stirring. An inert atmosphere of nitrogen gas was maintained. The reaction was carried out in a five liter round bottom flask in an oil bath. The temperature was maintained at 175°-180° C. for 45 minutes when the soap mixture was poured into three volumes of cracked ice. The viscous soaps were neutralized with cold concentrated hydrochloric acid. A white mass of solid fatty acid formed and was dissolved in Skelly F. This solution was washed with water, dried over sodium sulfate,

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[3] C. H. Dehydrated Castor Oil obtained through the courtesy of O. Eisenschmil, the Scientific Oil Company, Chicago, Ill.

freed of solvent, and stored for 48 hours at 36° C. The resulting white crystals were collected on a 90 mm Buchner funnel and recrystallized twice from both Skelly F and 95 percent ethyl alcohol. The product had a melting point of 55° C., had no perioxde value and possessed an absorption value (specific alpha value) of 110 at 2320 Å. This acid was preserved by suspension in ethyl alcohol and stored at -22° C. The white crystals were filtered from the solution as needed.

The liquid acids were kept pure by storing at -22° C. in sealed evacuated tubes.

Determination of Physical Constants

The physical constants used in following the course of autoxidation of linoleic acid were the peroxide number and the absorption value. The Wijs (62) iodine value served to indicate the purity of the acid used. The peroxide number was determined by a modified method of Wheeler (60) and the ultraviolet absorption value by the method of Mitchell et al. (49).

The iodine value was determined as follows: Approximately 0.1 g of fat was accurately weighed into a glass stoppered 125 ml flask followed by the addition of 5 ml of chloroform. Then 15 ml of Wijs solution was added with volumetric pipette. The flask was stoppered tightly and placed in the dark for exactly one hour. Ten ml of 15 percent potassium iodide was added and mixed well. The stopper and sides of the flask were washed

down with 5 ml distilled water. The solution was titrated with 0.1N sodium thiosulfate solution to a faint yellow, a few drops of starch indicator were added, and the solution titrated to clear white. Two blank samples containing no fat were titrated along with each group of unknowns.

The Wijs solution was made as follows: To one liter of acetic acid 13 g of iodine were added and heated until dissolved. When cold, chlorine gas was bubbled through the solution until a light orange-red color developed.

To make the sodium thiosulfate solution, 24.8 g of the solid thiosulfate was dissolved in 1000 ml of distilled water.

The 15 percent potassium iodide solution was made by dissolving 15 g of solid potassium iodide in 85 ml of distilled water.

To obtain the indicator, one g of soluble starch was boiled in 200 ml of distilled water for 10 minutes, at which time it was transferred to a sterilized bottle fitted with a medicine dropper.

In standardizing the thiosulfate solution, the following procedure was utilized: Ten ml of exactly 0.1N potassium dichromate and 5 ml of concentrated hydrochloric acid were placed in a 125 ml flask. Ten ml of 15 percent potassium iodide was added and the mixture titrated to a green color with thiosulfate solution. Three drops of starch indicator were added and the titration continued to a clear solution. The

calculations follow:

$$\frac{(126.9)\ (.1 \text{ for N of } K_2 Cr_2 O_7)}{(\text{ml of thio used in standardization})} = \text{Normality factor}$$

$$\frac{(\text{Normality Factor})\ (\text{Blank titration minus sample titration})}{(\text{Sample weight in g})} = \text{Iodine number}$$

The peroxide number was determined by the following method: A 0.1 g sample was accurately weighed into a 125 ml glass stoppered flask and dissolved in 10 ml chloroform-acetic acid. One ml of a saturated potassium iodide solution was added from a pipette. The flask was stoppered securely and shaken for exactly one minute. The stopper and side of the flask were was ed down with 5 ml of distilled water. A few drops of starch indicator were added and the mixture immediately titrated with .01N thio until chalky white.

The 0.01N thiosulfate was prepared by accurately diluting the 0.1N solution and standardized with 0.1N dichromate.

The saturated potassium iodide solution, which was made up fresh and kept in the dark, was obtained by placing 31 g of the solid in 20 ml distilled water.

The chloroform-acetic acid was prepared by intimately mixing 300 ml of concentrated acetic acid with 150 ml of redistilled chloroform.

The necessary calculations follow:

$$\frac{0.1 \text{ (for N of } K_2Cr_2O_7) \quad (2)}{\text{(ml of thio used in standardization)}} = \text{Normality of thiosulfate}$$

$$\frac{\text{(ml of titration)} \quad \text{(Nor. of Thio)} \quad (0.5) \quad (1000)}{\text{(Sample weight in g)}} = \text{Peroxide number}$$

Ultraviolet Absorption Measurements. The instrument used in these measurements was a model DU Beckman quartz spectophotometer. All determinations were made on alcoholic solutions as follows: one-tenth g was accurately weighed into a 100 ml volumetric flask. The sample was diluted to volume with redistilled absolute, aldehyde free alcohol and mixed well. A 10 ml aliquot was taken from the 100 ml flask and diluted to 250 ml. If at this dilution the sample was "too dark" to read, a 10 ml aliquot was transferred to a 50 ml volumetric flask and diluted to volume. Blanks and samples were diluted the same way. The sample was placed in the Beckman, balanced against the blank, and read at 2320 Å, 2340 Å and 2770 Å. The reading at 2340 Å was used in calculating the diene conjugation, and the one at 2770 Å served to indicate the presence and relative quantity of diene ketones.

The effects of alkali induced changes on the spectral absorption at various stages of oxidized linoleic acid was noted, Table 4. This technique was a modification of Lundberg's (46) method: To 27 ml of the alcohol solution read above, was added 3 ml of a 5 percent potassium hydroxide solution in 50 percent aldehyde free alcohol and the absorption

measurements made after 48 hours. The potassium hydroxide solution served to enolize any diene ketones present thus increasing the absorption value at 2770 Å. Sample calculations follow:

$$\text{Specific alpha} = \frac{\log_{10} \frac{I_o}{I}}{C\ L}$$

Where:

alpha = Absorption coefficient

I_o = Intensity of the radiation transmitted by the solvent

I = Intensity of radiation transmitted by the solution

C = Concentration of solute in g per 1000 ml

L = Length in centimeters of solution through which radiation passes

Example:

Reading = 0.900

Concentration = 0.900 gm/100 ml

Then diluted 10 to 250 ml

Then concentration/liter = $\frac{1000}{250}\ \frac{10}{100}$ Sample Wt.

and C = (0.4) (0.0900) = g/1000 ml

L = 1 cm

$$\text{alpha} = \frac{0.900}{(0.4)\ (0.900)} = 25$$

Oxidation Procedure. The oxidations were conducted by bubbling tank oxygen through heated samples for various known periods of time. From 5 to 6 g portions of sample were weighed into six tared, six inch, Pyrex lipless test tubes, were clamped into the apparatus as shown in Plate I. The oxygen

EXPLANATION OF PLATE I

A. The six inch Pyrex test tubes clamped in position with the oxygen entering inlet tubes.

B. The mechanical stirring unit.

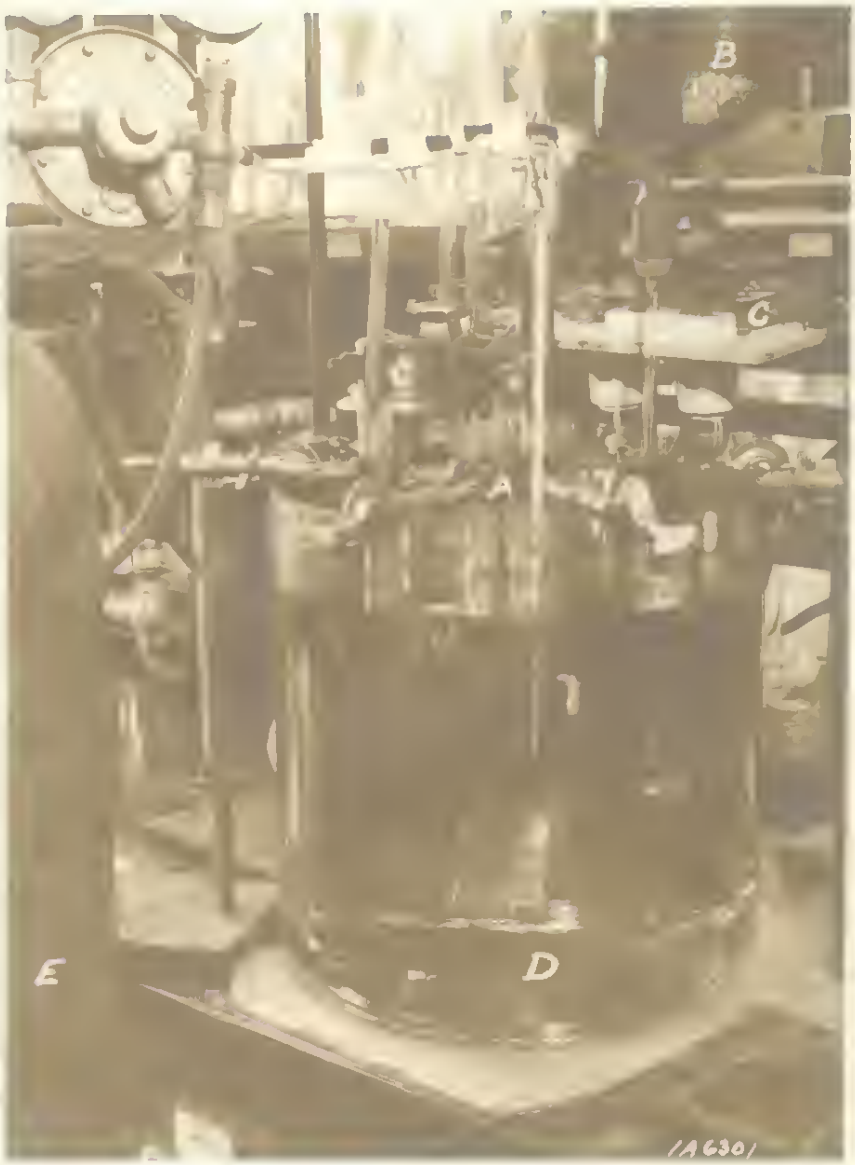
C. The Aminco thermostatically controlled heating unit.

D. The oil bath.

E. The oxygen tank.

(Bath insulation not shown)

PLATE I



was introduced into the sample by using drawn soft glass inlet tubes connected to the tank by rubber tubing as shown. A 12 inch by 12 inch Pyrex jar containing light mineral oil was used as a heating bath. The temperature was regulated to a constancy of ±0.5° C. by a Cenco stirrer and on Aminco thermostatically controlled heating unit. The flow of oxygen was regulated by a diaphragm gauge and a series of screw clamps between inlet tubes and tank.

Most of the samples were oxidized at 90 to ±0.5° C. (Figs. 1, 2, 5, 6, 7, 8, 9, 10), others were carried out at 65° C. (Tables 1, 2, 3). In one case Fig. 10), the tubes were cooled to room temperature at appropriate intervals and weighed. Two 0.1 g samples were removed for peroxide and spectrophotometric determinations, and the tubes reweighed and returned to the oxidation apparatus.

In regard to driers or inhibitors, five to six mg of foreign material was accurately weighed into the empty tube and the weight of added sample adjusted accordingly.

When not being oxidized, all samples were stored in an air tight container under nitrogen at -22° C.

KEY

1 UNCONJ. LINOLEIC ACID

2 CONJ. LINOLEIC ACID

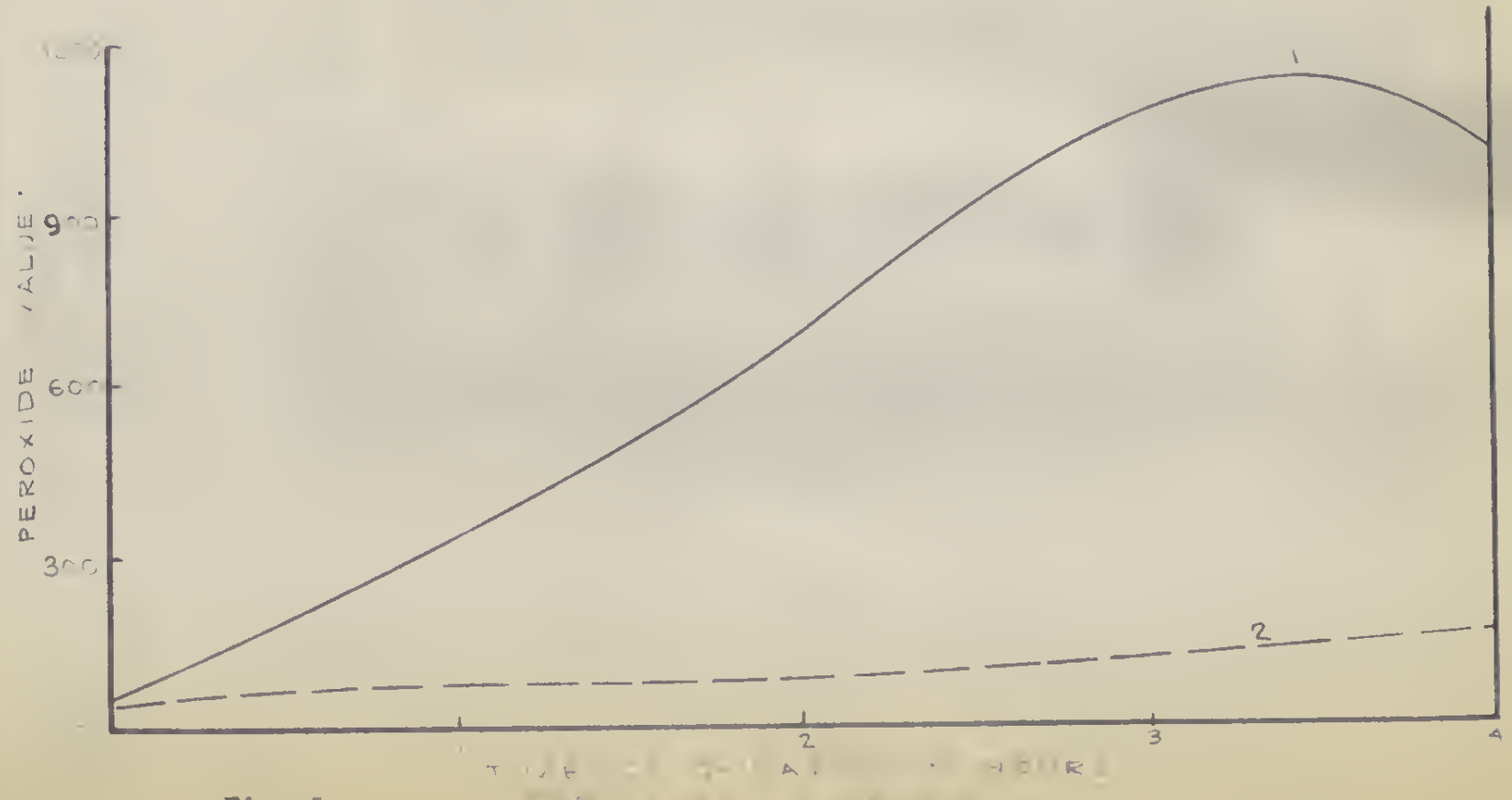


Fig. 1.

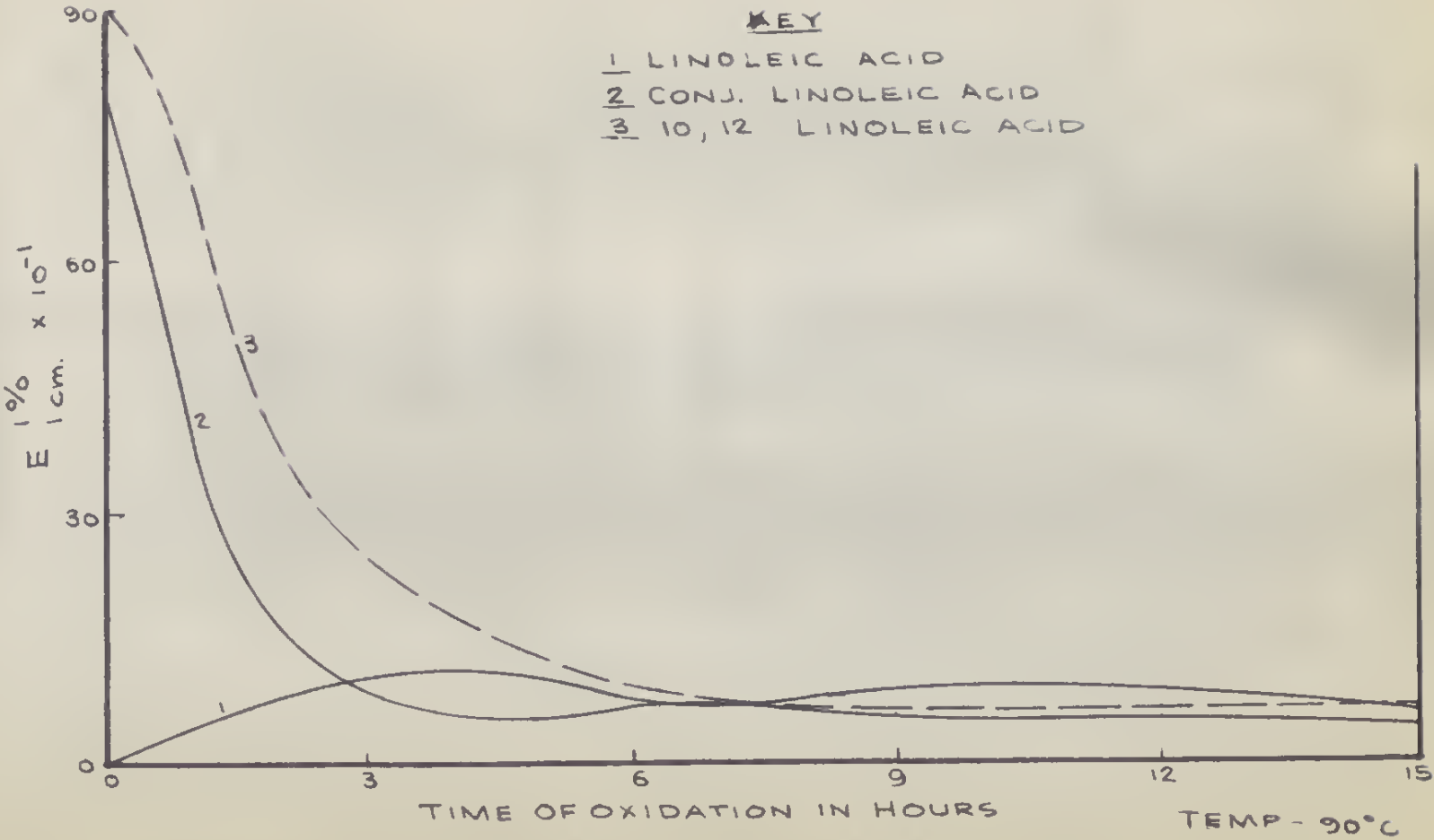


Fig. 2. ABSORPTION COEFFICIENT CURVES

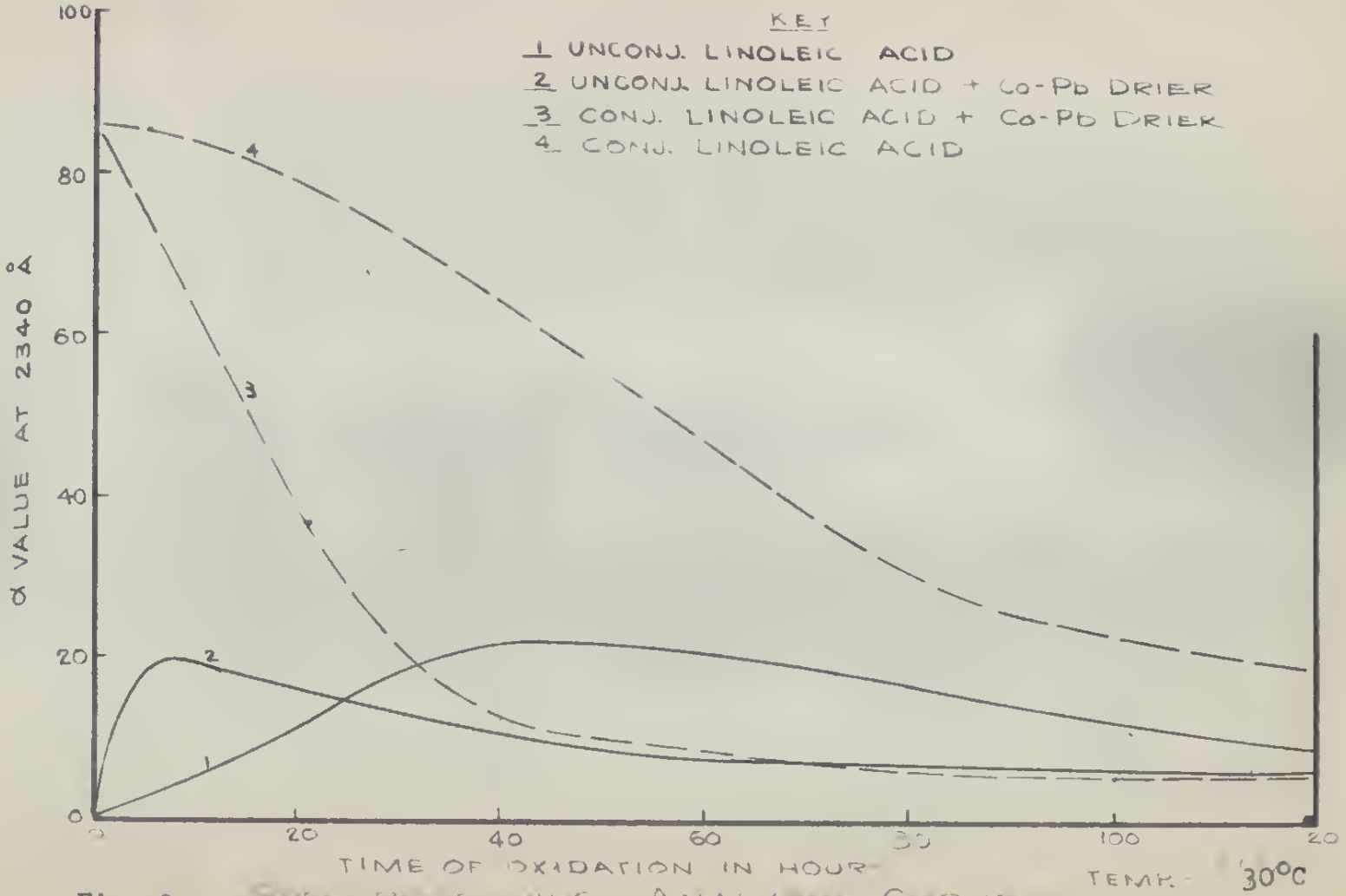

KEY
1 UNCONJ. LINOLEIC ACID
2 UNCONJ. LINOLEIC ACID + Co-Pb DRIER
3 CONJ. LINOLEIC ACID + Co-Pb DRIER
4 CONJ. LINOLEIC ACID
K VALUE AT 2340 Å
100
80
60
40
20
0
20
40
60
100
TIME OF OXIDATION IN HOUR
TEMP. 30°C

Fig. 3.

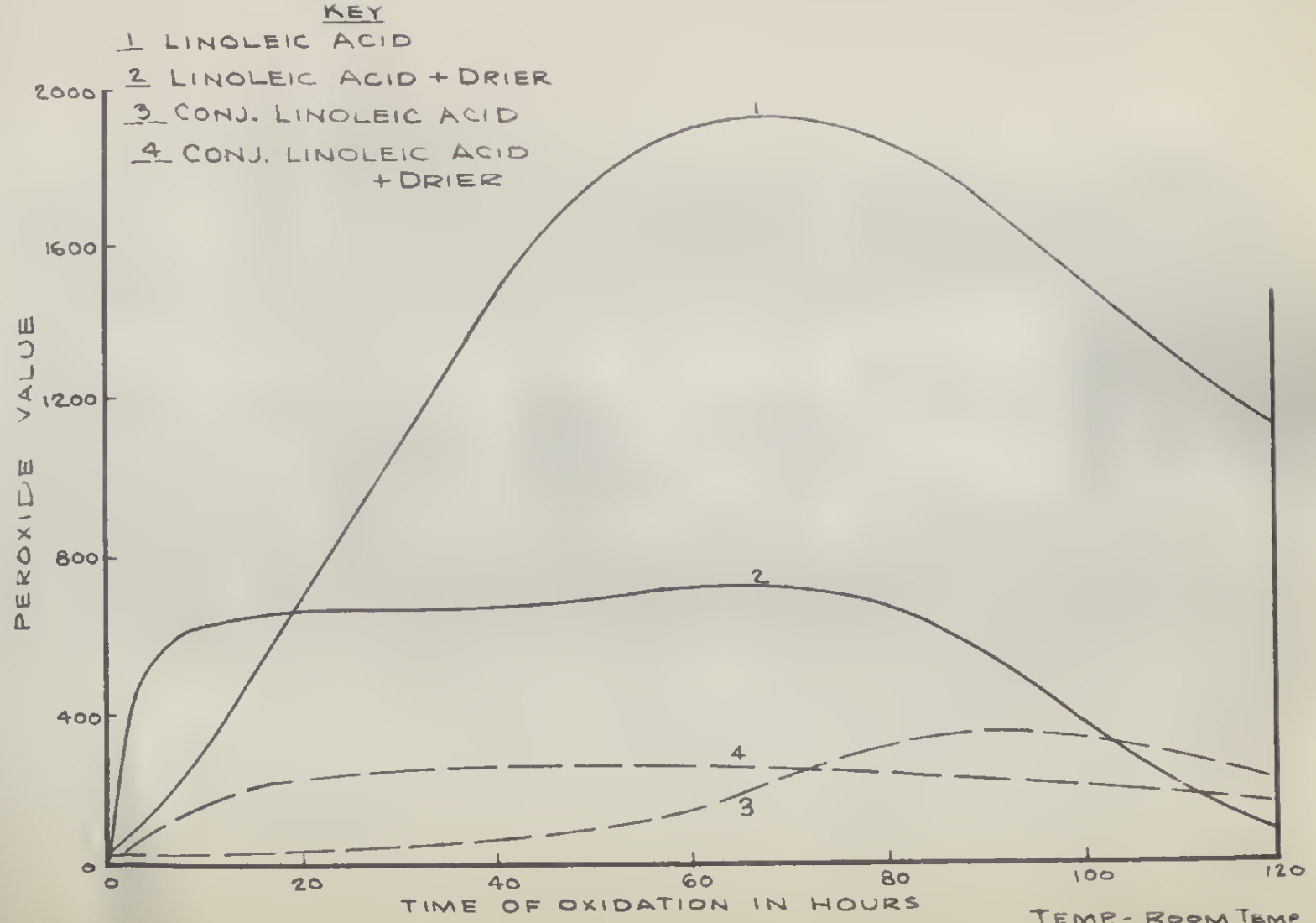


Fig. 4. PEROXIDE CURVES

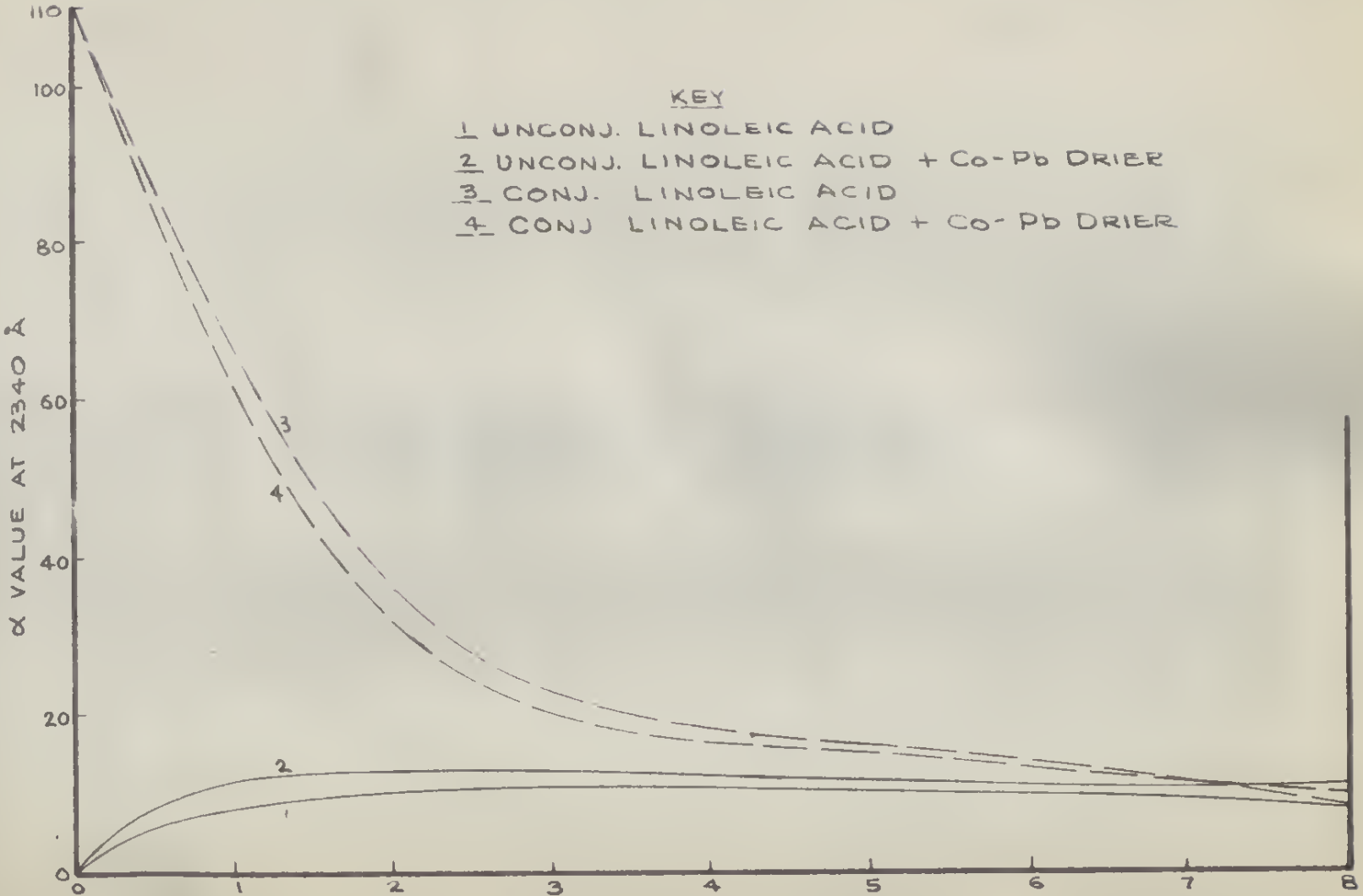


Fig. 5. SPECTROGRAPHIC ANALYSIS CURVES

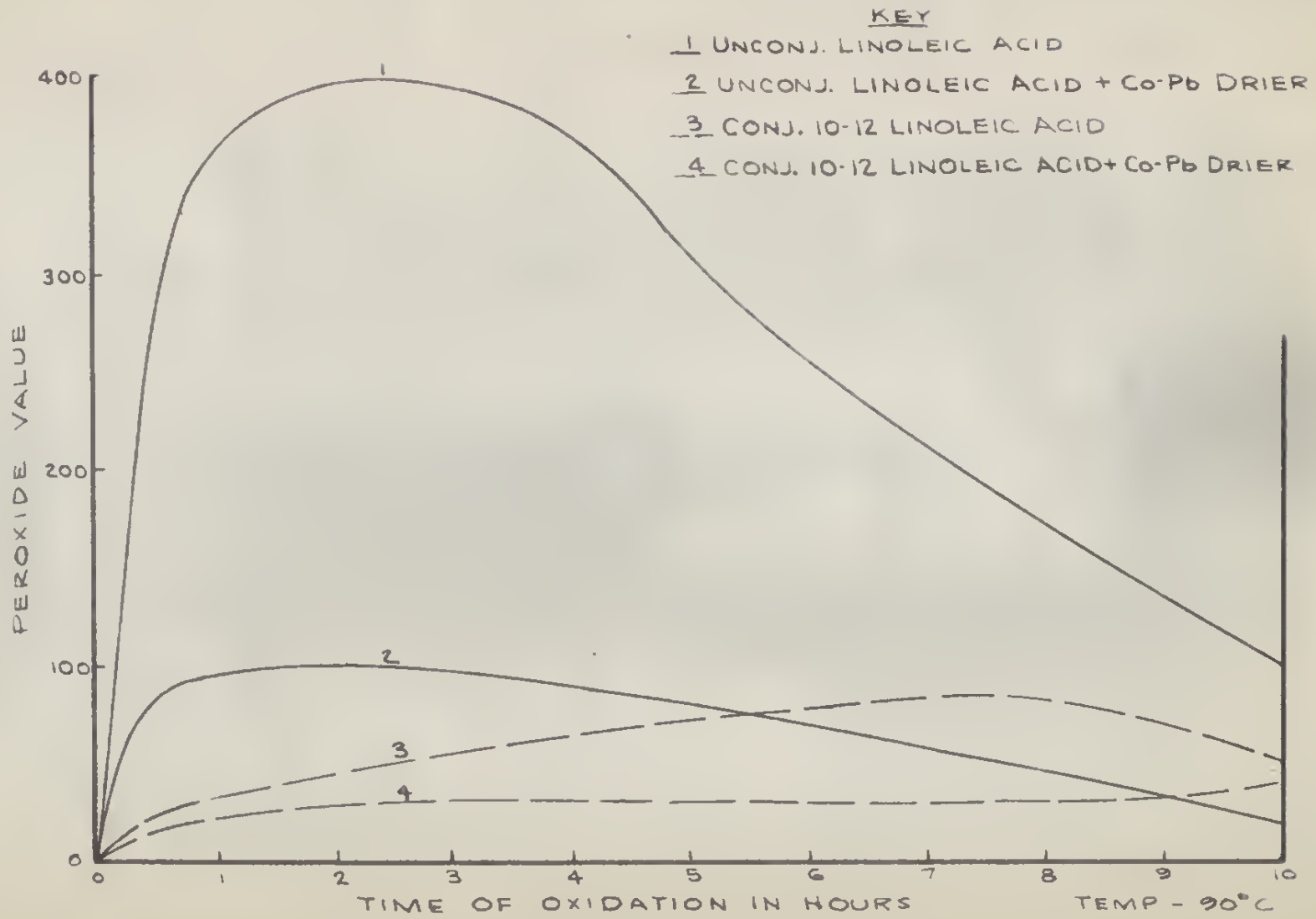


Fig. 6. PEROXIDE CURVES

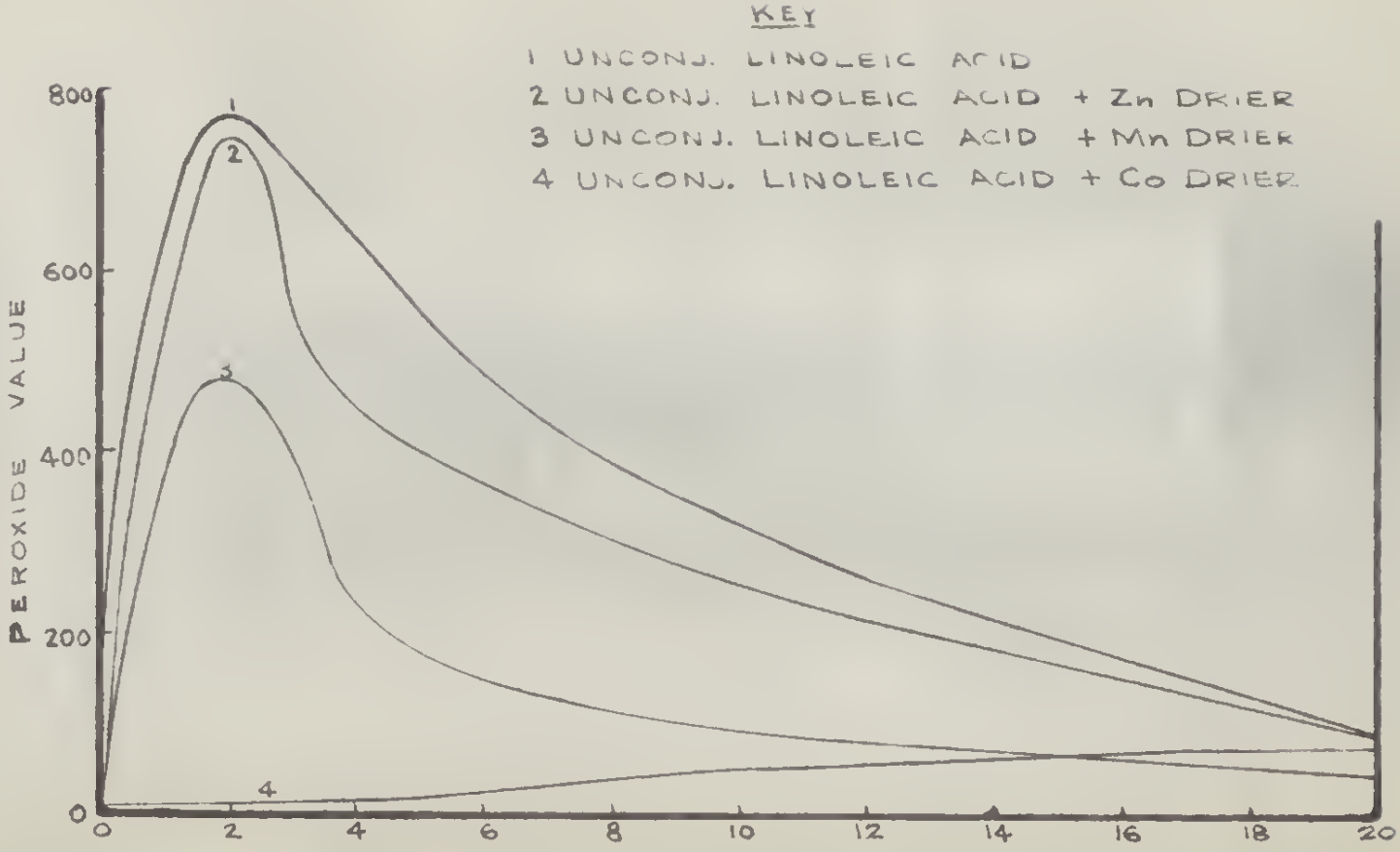


Fig. 7 PEROXIDE CURVES

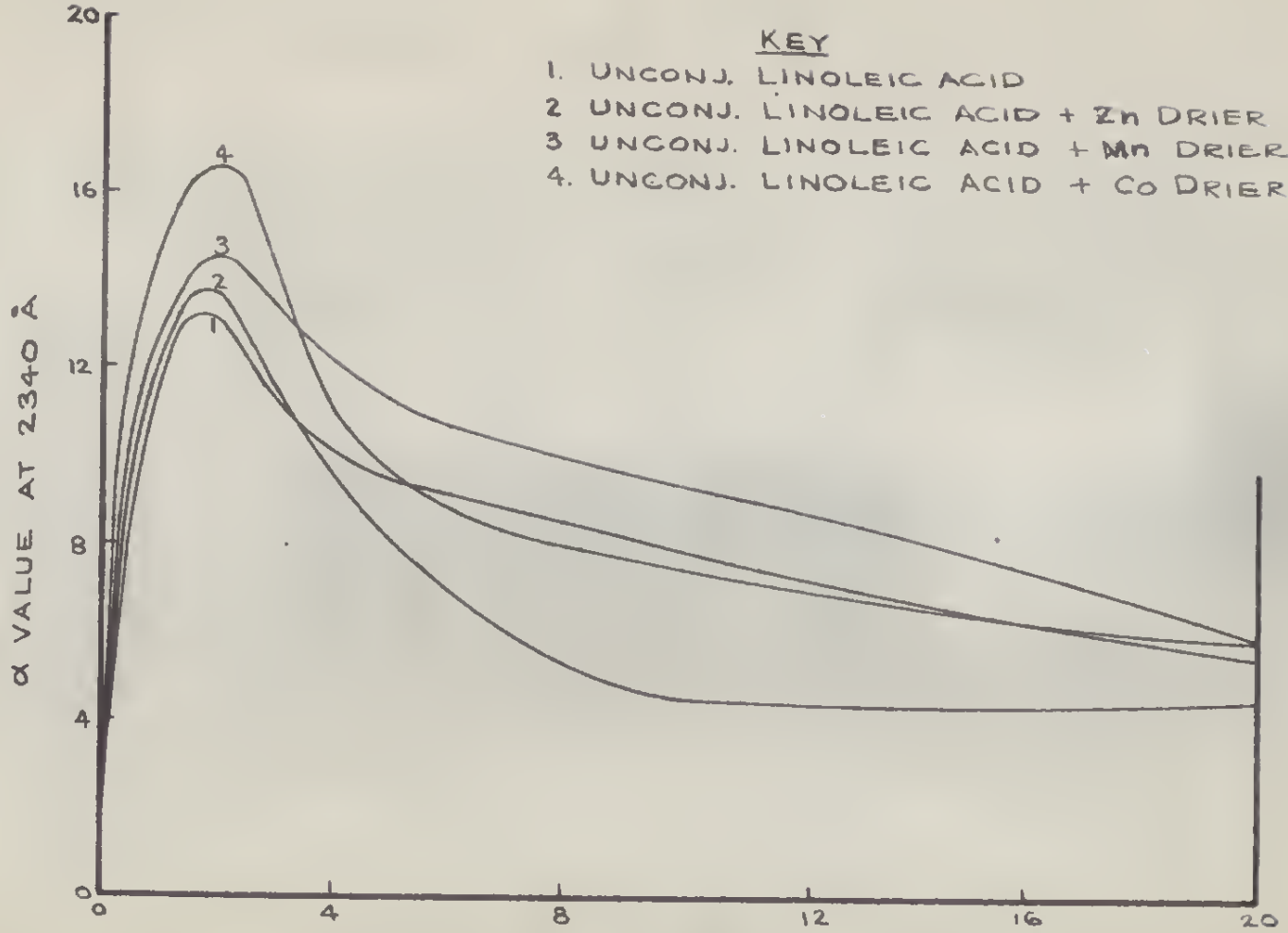


Fig. 8. SPECTROGRAPHIC ANALYSIS CURVES

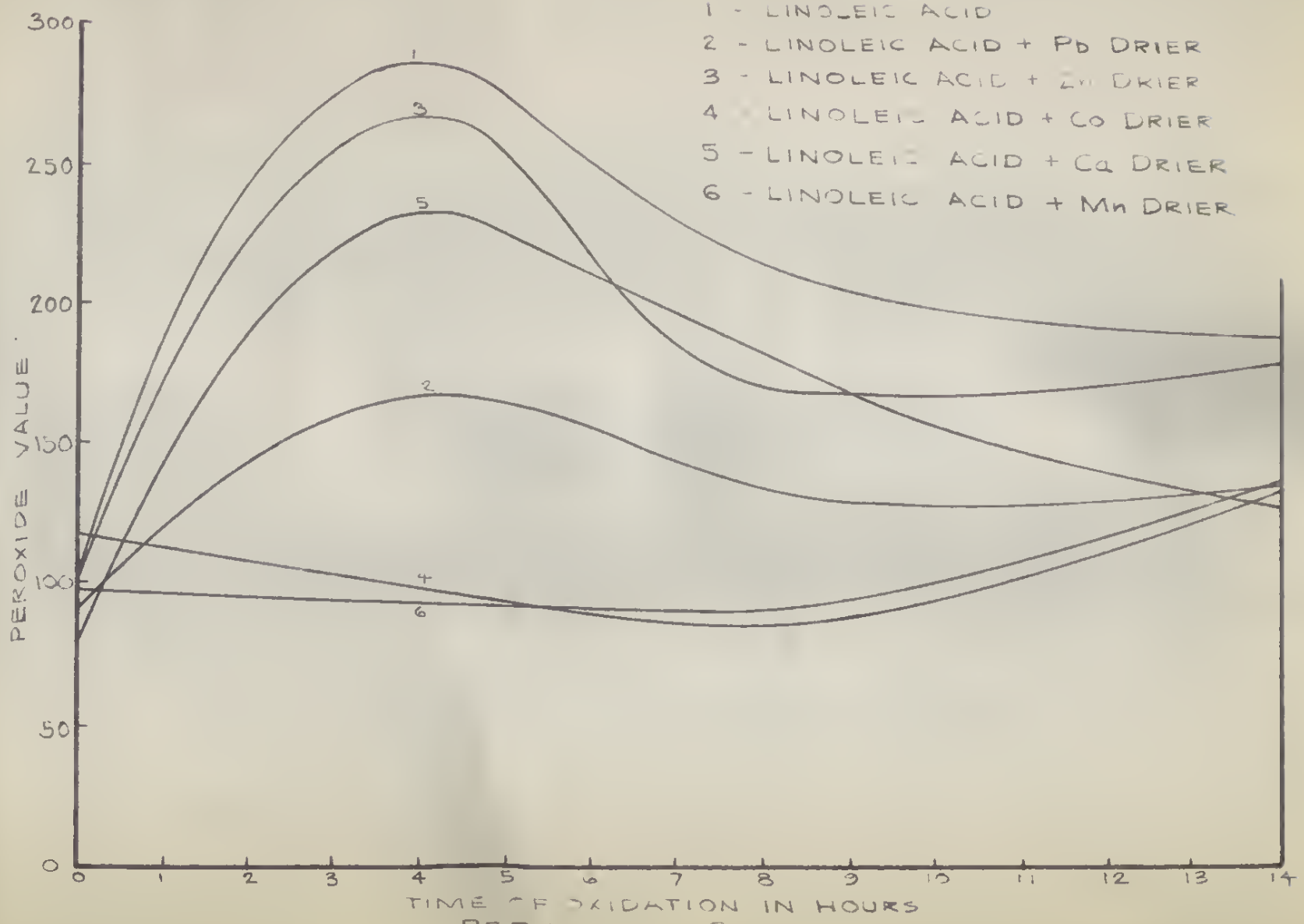


Fig. 9. PEROXIDE CURVES

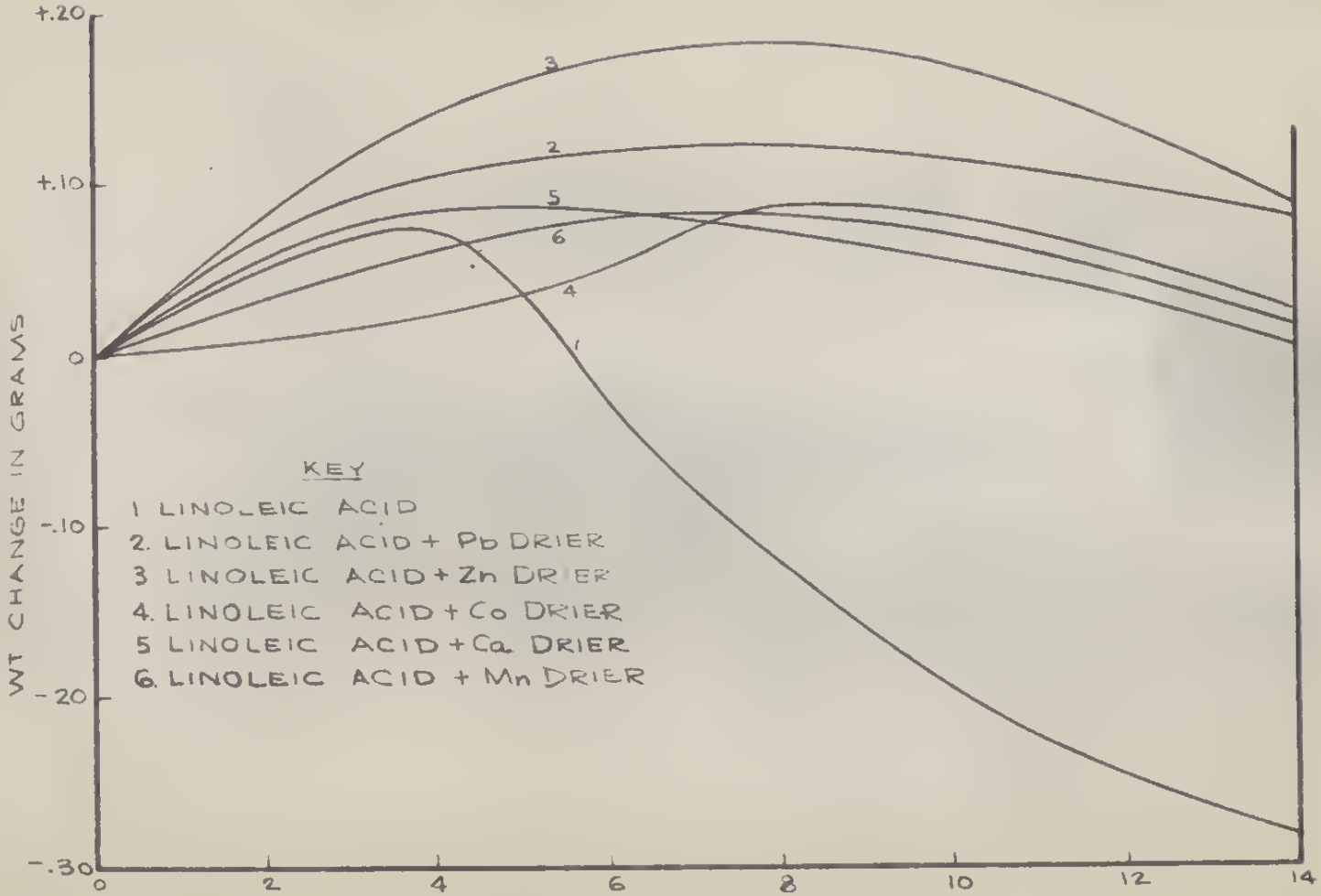


Fig. 10. WEIGHT CHANGE CURVES

Table 1. The oxidative course of unconjugated linoleic acid in the presence of inhibitors.

Time in hours	Linoleic acid unconjugated		Linoleic + alpha-tocopherol		Linoleic + p-dimethyl-amino-azo-benzene		Linoleic + hydroquinone		Linoleic + β-carotene		Linoleic + benzoyl peroxide	
	P.V.[1]	alpha-V[2]	P.V.	alpha-V	P.V.	alpha-V	P.V.	alpha-V	P.V.	alpha-V	P.V.	alpha-V
1-1/4	422	8.9	33	0.8	41	7.0	28	0.7	430	8.7	453	8.9
2-1/2	827	13.2	141	3.0	137	12.9	20	-	790	16.1	687	13.9
3-3/4	853	13.4	428	7.0	910	15.6	29	0.5	968	13.0	690	11.7
5	834	13.6	738	6.7	846	13.8	20	0.6	862	13.5	728	11.5
7-1/4	777	13.0	848	14.7	893	13.6	752	13.3	788	12.3	762	13.1
8-3/4	740	11.9	795	13.3	848	11.4	1159	16.1	783	10.7	742	11.6
11	810	10.8	890	12.8	828	9.1	1082	13.6	850	9.2	710	13.6
13	747	9.2	860	11.2	738	9.0	1012	12.0	900	8.0	690	10.3
15	705	8.3	785	9.1	668	8.6	843	10.4	608	7.6	670	8.9
20	503	5.6	628	6.3	554	6.7	598	7.7	500	5.8	522	6.2

[1] Peroxide value
[2] Absorption value at 2340 Å

Table 2. The oxidative course of 10, 12 linoleic acid in the presence of inhibitors.

Time in hours	10, 12 Linoleic acid		10, 12 Linoleic + alpha-tocopherol		10, 12 Linoleic + p-dimethyl-amino-azo-benzene		10, 12 Linoleic + hydroquinone		10, 12 Linoleic + β-carotene		10, 12 Linoleic + benzoyl peroxide	
	P.V.[1]	alpha-V[2]	P.V.	alpha-V	P.V.	alpha-V	P.V.	alpha-V	P.V.	alpha-V	P.V.	alpha-V
1-1/4	44	82	18	94	26	82	12	85	51	81	80	79
2-1/2	99	70	19	89	62	73	26	81	85	66	85	68
3-3/4	114	-	26	-	68	-	32	-	100	-	102	-
6	154	37	69	76	115	48	107	64	128	46	115	48
8	167	-	74	-	107	-	102	-	112	-	110	-
10	183	-	85	-	96	-	112	-	122	-	138	-
12	198	9	85	54	109	29	132	41	132	29	150	26
21	186	8	121	7	148	7	146	14	145	7	218	7
39	83	4	130	4	120	4	142	4	137	4	127	4

[1] Peroxide value

[2] Absorption value at 2340 Å

Table 3. The relation of peroxide content to absorption value at 2340 Å and to secondary enolizable products of autoxidation as indicated by the absorption at 2770 Å.

Time in hours	Unconjugated linoleic acid				Unconjugated linoleic acid + Co-naphthenate				10, 12 Linoleic acid				10, 12 Linoleic acid + Co-naphthenate			
	P.V.[1]	A[2]	-V[3]	KOH -V[4]	P.V.	A	-V	KOH -V	P.V.	A	-V	KOH -V	P.V.	A	-V	KOH -V
0.0	3	0.4	0.00	-	3	0.4	0.00	-	1	100.0	0.00	-	1	100.0	0.00	0.00
1.0	331	6.2	0.18	0.76	240	13.2	1.03	0.77	44	88.0	0.00	0.00	10	81.8	0.36	0.00
2.0	636	13.7	0.31	2.37	102	16.0	1.27	1.35	66	81.3	0.00	0.00	9	74.0	0.15	0.00
4.0	756	17.3	1.02	1.93	117	17.1	1.83	3.82	96	63.8	0.85	0.00	12	51.2	0.71	0.00
8.0	589	13.5	1.56	0.87	81	13.9	1.72	1.22	153	29.2	0.65	0.33	17	19.2	0.58	0.66
13.0	538	12.8	1.58	2.56	81	10.5	1.47	2.72	243	11.0	0.57	1.89	33	8.4	0.58	1.85
20.0	450	10.7	1.13	2.72	85	7.8	1.09	2.68	305	5.5	0.39	1.64	59	4.6	0.69	1.70
29.0	399	7.1	0.88	2.27	47	6.3	1.17	2.85	91	5.3	0.54	2.16	47	4.4	0.96	2.18

[1] Peroxide value
[2] Absorption value at 2340 Å
[3] Absorption value at 2770 Å
[4] Absorption value at 2770 Å, 48 hours after adding 5 per cent KOH

RESULTS

In the very early stages of autoxidation at 90° and 65° C., oxygen was rapidly absorbed by both the nonconjugated and conjugated linoleic acid, Figs. 1-9.

There were, however, three characteristics that indicated that oxidations of the nonconjugated acid seemed to involve the alpha-methylene group whereas oxidation of the conjugated acid seemed to involve the double bonds.

1. At the same rate of oxidation the peroxide content of the nonconjugated acid rose to eight times that of the conjugated acid which contains the same number of double bonds and adjacent methylene groups.

2. R. R. Allen[4] found that both the nonconjugated and conjugated acids could be completely hydrogenated before being subjected to oxidation. In addition, though the nonconjugated acid still absorbed nearly two moles of hydrogen per mole of acid after four hours of oxidation, the conjugated acid absorbed less than one mole after three hours and only a fraction of a mole after four hours of oxidation. This seems to indicate that regardless of the large peroxide formation the double bonds in the unconjugated acid were not blocked by the hydroperoxide group. On the other hand, it would appear that the double bonds in the conjugated acid were blocked by what was seemingly non-peroxide oxygen.

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[4] Private communication.

3. The ultraviolet absorption measurements indicated that the double bonds were free to shift in the nonconjugated, but not in the conjugated acid (Figs. 2, 3, 5, 8). In agreement with Hilditch (40) and others (46), a direct relationship between peroxide value and absorption in the region of 2325 Å was observed. The absorption coefficient of the nonconjugated acid increased rapidly but started to decrease before the peak in peroxide was reached. On the other hand, the absorption coefficient of the conjugated acid decreased rapidly before the formation of appreciable amounts of hydroperoxide.

The addition of alkali to the alcoholic solutions of peroxidized unconjugated acid caused a sharp increase in the absorption value in the region of 2770 Å even in the early stages of oxidation (Table 3). On the other hand, a sharp increase in this region was not observed until more than 70 percent of the conjugation had been destroyed in the case of the 10-12 conjugated acid (Table 3). The absorption at 2320 Å was decreased by alkali addition to the alcoholic solutions of both acids regardless of the extent of oxidation.

After the peak in peroxide formation had been reached, the addition of oxygen to both nonconjugated and conjugated acids seemed to follow the same pattern.

The height of the peroxide curve was profoundly influenced by the addition of commercial paint driers. These metallic naphthenates decreased the observed peroxide value of the nonconjugated and conjugated acids (Figs. 4, 6, 7, 9). Furthermore,

the absorption spectra at 2320 Å indicated that the addition of drier to the nonconjugated acid increased the absorption during the early stages of oxidation at 90°, 30° and 65° C. The addition of drier to the conjugated acid seemed to accelerate destruction of diene conjugation at 65° C. (Table 3). In regard to diene ketone formation, cobalt naphthenate appeared to increase the formation of this secondary product in both nonconjugated and conjugated samples (Table 3).

When the oxidation was carried out at room temperature (30° C.) (Figs. 2, 3), the type of peroxide curve obtained was approximately the same as when it was carried out at 90° C. It took only a longer period of time to reach a maximum hydroperoxide formation, and a longer period of time before changes in the ultraviolet absorption spectra were observed. The maximum peroxide value at room temperature was found to be 1900 instead of 800 and occurred after 80 instead of 4 hours of oxygenation.

The addition of 0.1 percent inhibitor to the unconjugated acid served only to lengthen the induction period as was expected (Table 1). The organic pro-oxidants added appeared to have a mild accelerating effect but did not greatly affect the hydroperoxide group itself (Table 1).

In the case of the conjugated acid neither the inhibitors nor the organic pro-oxidants exerted any clear cut influence on the oxidative course. It is, however, indicated that the inhibitor retarded the destruction of diene conjugation (Table 2).

DISCUSSION

The work in this laboratory serves to indicate further that the alpha-methylene groups are intimately and primarily involved in the autoxidation of nonconjugated linoleic acid. In agreement with Farmer (32), it was found that the oxidation of a conjugated olefin primarily involves the double bonds.

As Bolland and Gee (9) stated:

> In cases where a very high yield of alpha-methylenic hydroperoxide is obtained the argument that the oxidation starts at this point on that evidence alone is invalid unless the chain length of the oxidation is extremely short. A chain length of 100 is found in ethyl linoleate and 99% of the peroxide arises from the chain and therefore gives us no indication as to the nature of the primary product.

The data collected on antioxidants in this laboratory do not appear to be significant enough to warrant discussion (Tables 1 and 2).

The "initial catalytic" role of benzoyl peroxide (Tables 1 and 2) has been described by Robertson and Waters (54). The data represented in Figs. 4, 6, 7, 9 and Table 3 indicate that driers promote the decomposition of hydroperoxides during the autoxidation of nonconjugated linoleic acid but have much less noticeable effect upon the oxidation of the conjugated isomer.

Robertson and Waters (54) have shown that the early oxidation of the $-CH_2-$ group of tetralin is very much like that of the alpha- $-CH_2-$ in olefines. In addition they classified the cations of transition metals as "secondary catalysts"; viz.,

those which catalyzed the decomposition of the hydroperoxide.

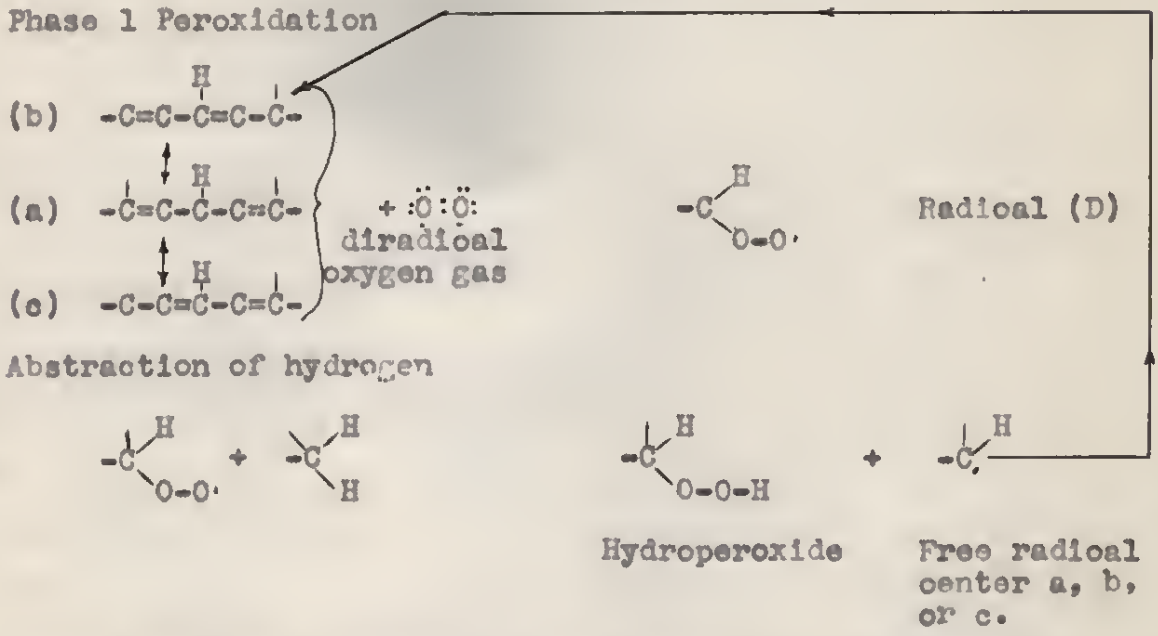
In this light it is fair to say that driers function as decomposition catalysts during the early oxidation of unsaturated fats. This role of driers would facilitate the destruction of any antioxidants present in a drying oil by providing active oxygen or other free radicals.

The observation of Morrell and Phillips (50) that cobalt drier gives a shorter chain than lead might possibly be correlated with the fact that the former is more efficient in promoting peroxide decomposition (Figs. 5, 7, 9). The new radicals thus formed serve to initiate secondary chains in effect causing the accelerated completion of the auto-catalytic phase and greater promotion of polymeric chains.

The apparent free radical nature of peroxide decomposition, the diradical form of oxygen gas (51) and evidence indicating that oxidation requires the prior formation of a free methylenic radical excludes the oxygen activation role of metallic driers in linoleic acid. This is in agreement with the work of Robertson and Waters (54), Yamada (61) and Medvedev (48) on tetralin. On the other hand, the alkali enolization of drier catalyzed samples did not indicate a large amount of ketone formation as would be expected if the hydroperoxides of linoleic acid decomposed exactly as that of tetralin. If the hydroperoxide splits at the -O-O bond, the failure to detect large ketonic formations may support Farmer's view that the hydroperoxide decomposes to form OH and O .

It seems possible that, exoluding the initial attack, oxygen aots upon the alpha-methylenic radical as follows:

Phase 1 Peroxidation



After the first resonating free radicals are produced by hydrogen abstraction, the diradical oxygen attacks to form radical (D) which takes over the abstraction role.

Since even so-called "pure" linoleic acid contains a minute amount of peroxide, it is fair to say that the above represents the chief way in which hydroperoxides are built up. The prior abstraction of hydrogen to produce resonating free radicals which are attacked by oxygen explains the relation of peroxide built up to diene conjugation in 9, 12 linoleic acid.

Phase 2 may be said to consist primarily of the breakdown of hydroperoxides to give free radicals of various types. Some of the radicals seem capable of attacking double bonds, others alpha-methylenic hydrogen. Farmer (32) has suggested that active oxygen (radical in character) might be given off as

follows:

$2\ ^{\#}OOH \longrightarrow H_2O + 2\ O$ -----attacks double bond

or

$-CH-OOH \longrightarrow H_2O + O$-----attacks double bond

or

$HOO^{\#} \longrightarrow HO^{\#} - O$ -----attacks double bond

Walsh (59) has represented the hydroperoxide decomposition of hydrocarbons as follows:

$-C-OOH \longrightarrow -C=O + {}^{\#}OH$ attacks double bond

Phase 3 seems to involve polymerization in which the oxygen added or the radicals produced in phase 2 can form a cyclic ether, a hydroxy ketone or a dimer by reacting with another linoleic acid radical.

These three phases appear to be in a state of flux during the autocatalytic period of the oxidation. Support for this view is found in the direct proportion of the peroxide values to the absorption values at both 2340 Å and 2770 Å, Table 3. Whereas the rise in the absorption at 2340 Å indicates the free radical nature of the peroxidation reaction the increased absorption at 2770 Å suggests that diene ketones are formed. These ketones would be enolized to triene structures by alkali.

At some point during this mixture of reactions the rate of hydroperoxide decomposition becomes greater than its formation. The alpha-methylenic radical content rapidly decreases, the detectable peroxides decline, and the complex and little understood polymerization reactions predominate.

To date, the hydroxystearic acid from the hydroperoxide at C_{11} has not been isolated. However, the C_9 and C_{13} hydroxystearic acids have been isolated from the hydrogenation mixture of autoxidized linoleic acid by Bergstrom (4). Failure to isolate this C_{11} acid may be due to the high resonance energy of stabilization of the system -C=C-C-C=C-. This energy was calculated by Orr, according to Bolland and Gee (9), to be 30.4 K cal and indicates that the C_9 and C_{13} methylenic radicals will be preferentially attacked by oxygen.

Despite the differences of opinion concerning peroxide decomposition, addition of oxygen to the double bond, drier mechanism, and olefin polymerization, within the past 10 years much progress has been made toward the solution of the general problem of autoxidation.

When new data; viz., kinetic, spectroscopic, chemical and thermochemical, are correctly correlated and interpreted along with existing knowledge of oxidation, the exact mechanisms of autoxidation and polymerization reactions in unsaturated fats may be postulated.

SUMMARY

A comparison of various characteristics indicated that the autoxidations of nonconjugated and conjugated linoleic acid followed somewhat different courses.

The position of the double bonds seemed to determine at

what point attachment of molecular oxygen took place. When these bonds were conjugated oxygen added directly to them. When they were not conjugated, molecular oxygen added to the active methylene group.

Though diene conjugation paralleled hydroperoxide formation, it declined sooner.

The addition of commercial paint driers to nonconjugated acid increased the rate of diene conjugation during autoxidation and greatly lowered the amount of detectable peroxides. Conversely, the addition of driers to the conjugated isomer lowered the amount of detectable peroxide and mildly promoted the destruction of conjugation.

Metallic naphthenates slightly increased the quantity of diene ketones in both nonconjugated and conjugated acids though after a longer period of oxygenation in the case of the latter.

The preceding investigation indicates that the autoxidative reactions are in a continual state of flux. The stage of oxidation determines the predominant reaction, which in turn controls the characteristics of any sample removed for analysis.

ACKNOWLEDGMENT

The author wishes to express his appreciation for the help and advice given by Dr. F. A. Kummerow and the interest of Dr. D. B. Sharp. He is also indebted to Miss Margaret Seaton and Mr. Donald Beeson for their aid in carrying out the analytical work involved.

LITERATURE CITED

(1) Alyea, H. N. and H. L. J. Backstron.
The inhibitive action of alcohols on the oxidation of sodium sulfite. Amer. Chem. Soc. Jour. 51: 90-109. 1929.

(2) Atherton, D. and T. P. Hilditch.
Union of gaseous oxygen with methyl oleate at 20° and 120°. Chem. Soc. Jour. p. 105. 1944.

(3) Backstrom, H. L. J.
The chain reaction theory of negative catalysts. Amer. Chem. Soc. Jour. 49: 1460-1472. 1927.

(4) Bergstrom, Suno.
Autoxidation of linoleic acid. Nature. 156: 717-718. 1945.

(5) Bodenstein, M.
Eine theory der photochemischen reactionsgeschwindigkeiten. Z. Physikol Chemie. 85: 329-397. 1913.

(6) Bolland, J. L.
The thermal oxidation of ethyl linoleate. Royal Soc. Proc. A. 186: 218-236. 1946.

(7) Bolland, J. L. and H. P. Koch.
Course of autoxidation reactions in polyisoprenes and related compounds. IX. The primary thermal oxidation product of ethyl linoleate. Chem. Soc. Jour. p. 445. 1945.

(8) Bolland, J. L. and Geoffrey Gee.
Kinetic studies in the chemistry of rubber and related materials. II. The kinetics of the oxidation of unconjugated olefins. Faraday Soc. Trans. 42: 236-243. 1946.

(9) Bolland, J. L. and G. Gee.
III. Thermochemistry and mechanisms of olefin oxidation. ibid. 42: 244-252. 1946.

(10) Bolland, J. L. and P. ten Have.
The inhibitory of hydroquinone on the thermal oxidation of ethyl linoleate. ibid. 43: 201-210. 1947.

(11) Christiansen, J. A. and H. A. Kramers.
Uber die geschwindekeiten chemischer reactionen.
Z. Physikol Chemie. 104: 451-471. 1923.

(12) Coffey, Samuel.
The action of driers in the oxidation of drying oils. Chem. Soc. Jour. p. 17. 1922.

(13) Cook, A. H.
Catalytic properties of the phtalocyanines II oxidase properties. Chem. Soc. Jour. p. 1768-1774. 1938.

(14) Criegee, R.
Intermediate products in dehydrogenations with quinones. Ber. 69B. 2758-2761. 1936.

(15) Criegee, R., H. Pilz and H. Flygare.
Olefin peroxides. Berichte. 72: 1799-1804. 1939.

(16) Curwen, M. D.
Naphthenate driers. Oil and Colour Trades Jour. 88: 1711. 1935.

(17) Ellis, G. W.
The chemistry of drying oils I. Soc. Chem. Ind. Jour. 44: 401-408T. 1925.

(18) Ellis, G. W.
The chemistry of drying oils II. ibid. 44: 463-468T. 1925.

(19) Ellis, G. W.
The chemistry of drying oils III. ibid. 44: 469-472T. 1925.

(20) Ellis, G. W.
Contribution to the chemistry of drying oils III. ibid. 45: 193-199T. 1926.

(21) Ellis, G. W.
Autoxidation of the fatty acids. I. The oxygen uptake of elaidic, oleic, and stearic acids. Biochem. Jour. 26: 791-800. 1932.

(22) Elm, A. C.
A century of progress in driers. Indus. and Engg. Chem. 26: 386. 1934.

(23) Engler, C. and W. Wild.
Ueber die sogenannte activirung des sauerstoffs und uber superoxydbildung. Berichte. 30: 1669-1681. 1897.

(24) Engler, C. and J. Weissberg.
Ueber activirung des sauerstoffs II. Der active sauerstoff des terpentinols. Berichte. 31: 3046-3055. 1898.

(25) Engler, C.
Ueber activirung des sauerstoffs VI. Demonstration der bildung des wasserstoffsuperoxyds und anderer superoxyde als products directer oxydation. ibid. 33: 1109-1111. 1900.

(26) Fahrion, W.
Theory of leather formation. Z. Angew. Chem. 22: 2083-2097. 1909.

(27) Farmer, E. H.
Methylenic reactivity in olefinic and polyolefinic systems. Faraday Soc. Trans. 38: 356-361. 1942.

(28) Farmer, E. H. and D. A. Sutton.
Autoxidation reactions in polyisoprenes and allied compounds. (II) hydroperoxide structure and chain scission in low mol. polyisophrenes. Chem. Soc. Jour. 119-122. 1943.

(29) Farmer, E. H. and D. A. Sutton.
IV Isolation and constitution of photochemically-formed methyl oleate peroxide. ibid. 122-125. 1943.

(30) Farmer, E. H., H. P. Koch and D. A. Sutton.
Autoxidation reactions in polyisoprenes and allied compounds VII rearrangement of double bonds during autoxidation. Chem. Soc. Jour. 541-547. 1943.

(31) Farmer, E. H., G. F. Bloomfield, A. Sundralingam and D. A. Sutton. Autoxidative reactions in olefines and polyolefinic systems, including rubber. Faraday Soc. Trans. 38: 348-356. 1942.

(32) Farmer, E. H.
Peroxidation in relation to olefinic structure. Faraday Soc. Trans. 42: 228-236. 1946.

(33) Filer, L. J., K. F. Mattil and H. E. Longenecker.
Antioxidant losses during the induction period of fat oxidation. Oil and Soap. 21: 289-292. 1944.

(34) Fokin, S.
Catalytic oxidation and reduction reactions of organic compounds. Z. Angew. Chem. 22: 1451-1459. 1909.

(35) Fokin, S.
Catalytic oxidation and reduction reactions of organic compounds. ibid. 22: 1492-1502. 1909.

(36) Fritz, F.
Driers and their use. Chem. Ztg. 60: 921-924. 1936.

(37) Golumbic, C.
The antioxygenic action of phosphoric acid in association with tocopherols and hydroquinones. Oil and Soap. 19: 181-182. 1942.

(38) Golumbic, C.
The antioxidative behavior of vegetable and animal fats. Oil and Soap. 20: 105-107. 1943.

(39) Greenfeld, S. M.
Naphthenate driers. Paint Manufacture. 5: 154-155. 1935.

(40) Hilditch, T. P. and F. D. Gunstone.
Union of gaseous oxygen with methyl oleate, linoleate and linolenate. Chem. Soc. Jour. 836-841. 1945.

(41) Hock, H. and W. Susemihl.
Autoxidation of hydrocarbons I. A tetrahydronaphthalene obtained by autoxidation. Berichte 66B. 61-68. 1933.

(42) Holman, R. T. and O. C. Elmer.
Rates of oxidation of unsaturated acids and esters. Amer. Oil Chemists Soc. Jour. 24: 127. 1946.

(43) Ivanov, K. I., V. K. Savinova and E. G. Mikhailova.
Effect of accelerators and inhibitors of autoxidation and the decomposition of organic peroxides. Physic. Chem. Jour. U.R.S.S. 12: 719. 1939.

(44) Kummerow, F. A. and Eileen L. Green.
The non-uniformity of -linoleic acid. Amer. Oil Chemists Soc. Jour. 24: 196-199. 1947.

(45) Kummerow, F. A., J. Hite and S. Kloxin.
Rancidity in eviscerated poultry. Poultry Sci. (In press).

(46) Lundberg, W. O. and J. R. Chipault.
The oxidation of methyl linoleate at various temperatures. Amer. Chem. Soc. Jour. 69: 833-836. 1947.

(47) Markley, Klare S.
Fatty acids, their chemical and physical properties. Interscience. 668 p. (p. 453) 1947.

(48) Medvedev, S. S.
Role of peroxides in the oxidation of hydrocarbons. Acta Physicochemica. U. R. S. S. 9: 395-420. 1938.

(49) Mitchell, J. H., H. R. Kraybill and F. P. Zscheile.
Quantitative spectrum analysis of fats. Indus. and Engg. Chem. Analyt. Ed. 15: 1-3. 1943.

(50) Morrell, R. S. and E. O. Phillips.
Autoxidation of drying oils. Paint Tech. 7: 130-132, 169-170, 187. 1942.

(51) Remick, Edward A.
Electronic interpretations of organic chemistry. New York. John Wiley. 474 p. (p. 272) 1943.

(52) Rieche, A.
The oxidation of organic compounds with air oxygen. Angew. Chem. 50: 520-524. 1937.

(53) Riemenschneider, Ray W.
Oxidative rancidity and the use of antioxidants. Amer. Assoc. Cereal Chem. Trans. V. 50-63. 1947.

(54) Robertson, Alan and W. A. Waters.
Some features of the oxidation of tetralin. Faraday Soc. Trans. 42: 201-210. 1946.

(55) Rollett, Adolf.
Linoleic acid. Z. Physiol. Chem. 62: 410-421. 1909.

(56) Standinger, H.
Autoxidation of organic compounds III. Autoxidation of asym. diphenylethlene. Berichte. 58B. 1075-1079. 1925. of C. A. 8: 685.

(57) Stephens, H. N.
Autoxidation I. Cyclohexene peroxide. Amer. Chem. Soc. Jour. 50: 568-571. 1928.

(58) Von Mikusch, J. D.
Solid 10-12 octadecadienoic acid.--new linoleic acid melting at 57° C. Amer. Chem. Soc. Jour. 64: 1580. 1942.

(59) Walsh, A. D.
Processes in the oxidation of hydrocarbon fuels.
Faraday Soc. Trans. 42: 269-283. 1946.

(60) Wheeler, D. H.
Peroxide formation as a measure of autoxidative deterioration. Oil and Soap. 9: 89-97. 1932.

(61) Yamada, T.
Decomposition velocities of organic peroxides.
Chem. Zentr. I. 3494. 1941.

(62) Yasuda, M.
The determination of the iodine number of lipids.
Biol. Chem. Jour. 94: 401. 1931.