[CONTRIBUTION FROM THE HORMEL INSTITUTE, UNIVERSITY OF MINNESOTA]

The Oxidation of Methyl Linoleate at Various Temperatures[1]

BY W. O. LUNDBERG AND J. R. CHIPAULT

For a long time it was considered that the first products formed in the autoxidation of fats, including linoleic acid and its esters, were ethenoid peroxides having cyclic peroxidic structures. In accordance with the theories of Rieche,[2] it has been shown by Farmer and his associates[3,4] within the last few years that under some conditions the primary products of autoxidation in unsaturated fatty acids are hydroperoxides. Recently Bolland and Koch[5] have found that the primary product in the autoxidation of methyl linoleate at 37° is a monohydroperoxide which was shown by spectrographic analysis to contain at least 70% of conjugated diene isomers resulting from a rearrangement of the unconjugated diene. They accounted for the observed displacement of double bonds by a free radical mechanism of oxidation, such as was postulated by Farmer and his associates.[6] According to this mechanism, three isomeric linoleate hydroperoxides are possible, two of which contain conjugated diene structures.

Bergström[7] independently arrived at the same conclusion regarding the development of conjugated diene hydroperoxides, and further strengthened the evidence by isolating 9- and 13-hydroxystearic acids from the products obtained upon the hydrogenation of autoxidized linoleate. He was unable to obtain any 11-hydroxystearic acid, which would be the corresponding hydrogenation product of the proposed unconjugated diene hydroperoxide. He also found that linoleate autoxidized at 37° contained peroxides which did not exhibit diene conjugation, and which on hydrogenation yielded alpha glycolic groups. Hydrogenation of samples taken in all stages of the autoxidation yielded a constant proportion of approximately 0.2 mole of alpha glycolic groups for every mole of oxygen that had been absorbed.

Atherton and Hilditch[8] found the oxidation of methyl oleate at 120° to be quite different from the oxidation at 20°, and indicated that at the higher temperature, oxygen reacted to a considerable extent with the ethenoid bond in accordance with the classical concept, yielding cyclic peroxides rather than hydroperoxides. Gunstone and Hilditch[9] studied the autoxidation of ethyl linoleate at 20, 50 and 80° and stated that at all of these temperatures, the development of conjugated diene unsaturation was observed to parallel the formation of peroxides.

The present paper describes results obtained in the autoxidation of methyl linoleate at 40, 60, 80 and 100°. The investigation has been confined to the early stages of the oxidation.

Experimental

Preparation of Methyl Linoleate.—The methyl linoleate was prepared from corn oil *via* the tetrabromides by conventional methods. Efforts were made to obtain a very pure product. The tetrabromostearic acids were repeatedly recrystallized to a melting point of 115.2°. From the debromination step to the time of introduction of the lineoleate into the autoxidation vessel, contact with oxygen was not permitted, all transfers being made under nitrogen that had been purified by passage over hot copper. The washed and dried methyl linoleate was further purified by distillation through a 25-mm., 100-plate Podbielniak Hypercal fractionating column.

The final product had a peroxide value of zero as measured by the method described below, a Wijs iodine number of 173.1 (theoretical 173.2), and a specific absorption after alkali isomerization[10] of 81.9, calculated on the basis of methyl linoleate, and 86.0 on the basis of linoleic acid. The amounts of conjugated diene, triene and tetraene calculated by the method of Brode, *et al.*,[11] were 0.039, 0.0005 and 0.00005%, respectively.

Oxygen Absorption Measurements.—The measurements were conducted at the several temperatures in a Warburg respirometer using 5-ml. samples in 70-ml. flasks of a vertical cylindrical type, having an inside diameter of approximately 5.5 cm. The flasks were shaken at 60 oscillations per minute through an amplitude of 2 cm. When the samples had attained previously selected levels of oxygen absorption, they were rapidly chilled, and spectrophotometric analyses and peroxide determinations were immediately performed.

At 40 and 60° the samples were equilibrated in the bath for ten minutes before closing the manometers. This was possible without introducing any serious errors in the oxygen absorption measurements since at these temperatures no measurable oxygen absorption occurred during that interval. At 80 and 100°, equilibration in this manner was not possible because an appreciable absorption of oxygen began almost at once. The samples were therefore equilibrated at 60°, the manometers closed, and the samples then introduced into the bath at 80 or 100°. A corrected initial pressure reading was then obtained by calculation from the flask volume and temperature change.

Determination of Peroxides.—In an effort to obtain highly reproducible and accurate peroxide values, a modification of the usual iodometric methods was developed in which all the oxygen was rigorously excluded during the critical phases of the determination. The procedure is as follows: 10 ml. of 2:1 acetic acid–chloroform is introduced into a 125-ml. Erlenmeyer flask, which is then covered with a dental rubber dam. A nitrogen inlet tube is introduced through the dam and nitrogen purified over hot

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[1] The subject matter of this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions and conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the War Department.

[2] Rieche, "Die Bedeutung der organische Peroxide," Stüttgart, 1936.

[3] Farmer and Sutton, *J. Chem. Soc.*, 119 (1943).

[4] Farmer, Koch and Sutton, *ibid.*, 541 (1943).

[5] Bolland and Koch, *ibid.*, 445 (1945).

[6] Farmer, Bloomfield, Sundralingam and Sutton, *Trans. Faraday Soc.*, **38**, 348 (1942).

[7] Bergström, *Arkiv Kemi, Mineral. Geol.*, *Bd.* **21A**, N:0 14.

[8] Atherton and Hilditch, *J. Chem. Soc.*, 105 (1944).

[9] Gunstone and Hilditch, *ibid.*, 836 (1945).

[10] Mitchell, Kraybill and Zscheile, *Ind. Eng. Chem., Anal. Ed.*, **15**, 1 (1943).

[11] Brode, Patterson, Brown and Frankel, *ibid.*, **16**, 77 (1944).

copper is bubbled through the solvent for five minutes to remove oxygen. The flask is placed in a constant temperature bath maintained at 35° and, after two minutes, the sample of oxidized methyl linoleate is introduced through the dam by means of a calibrated 1-ml. syringe. Two ml. of a freshly prepared saturated solution of potassium iodide in methyl alcohol (freshly distilled from potassium hydroxide and zinc dust) from which all oxygen has been removed by the passage of pure nitrogen are then added. After fifteen minutes, 10 ml. of water is added. At this stage the nitrogen inlet tube is removed from the flask and the liberated iodine immediately titrated with 0.01 *N* sodium thiosulfate. The entire procedure is so arranged that the contents of the reaction flask are not in contact with any appreciable amount of dissolved or atmospheric oxygen at any time until the water is added.

Under these conditions the liberated iodine rises to a maximum concentration in less than fifteen minutes, and thereafter remains constant. Reaction times up to two hours produce no changes from the peroxide values obtained with a reaction time of fifteen minutes.

Ultraviolet Absorption Measurements.—Techniques somewhat similar to but more refined than those described in other studies[12] were used in the spectrophotometric measurements. The ultraviolet absorption of autoxidized samples of methyl linoleate dissolved in appropriate concentrations in air-free alcohol was measured in the wave length vicinities of 2325 and 2775 Å. To 9 ml. of the alcoholic solutions was then added 1 ml. of a 5% potassium hydroxide solution in 50% alcohol (air-free), and the absorption measurements again made after various measured intervals. Other concentrations of potassium hydroxide were also used in the preliminary studies.

The effects of alkali-induced changes on the spectral absorption of autoxidized methyl linoleate in relation to time are shown in Fig. 1. The peroxide value of this sample was about 100 m. e./kg. At 2325 Å. there is a progressive and gradual decrease in absorption which is not entirely complete after forty-eight hours. The changes are greatest when the 5% potassium hydroxide solution is added, and other studies in which a still more dilute potassium hydroxide solution was used indicate that further decreases in concentration do not produce any appreciable increase in these changes.

At 2775 Å., there are increases in absorption upon the addition of alkali. If the early changes obtained in Fig. 1 are plotted on a magnified time scale, it is found that there is an immediate and almost instantaneous increase. This is followed by a more gradual and protracted increase, which reaches a maximum after forty-eight hours.

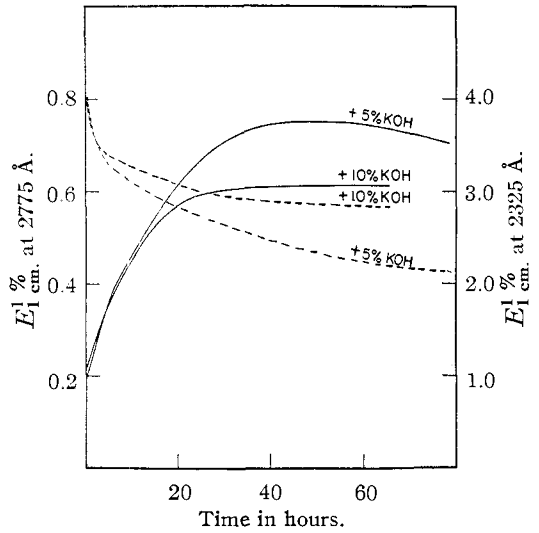

Fig. 1.—Changes in $E^{1\%}_{1\,\text{cm.}}$ with time in the presence of KOH: ----, 2325 Å.; —— 2775 Å.

Results and Discussion

Typical curves of the oxygen absorption in relation to time at various temperatures are given in Fig. 2. The induction periods shown in this figure are not necessarily any that were observed in actual measurements; the starting points of the curves on the horizontal axis have been selected arbitrarily in order to arrange the curves conveniently. The samples at 40 and 60°, however, actually showed induction periods which were not reproducible and which varied in duration from a few minutes to eight hours. During this period, no measurable oxygen absorption took place, and the samples maintained a peroxide value of zero. Following this period, there was an initial brief interval of relatively rapid oxygen absorption, the significance of which is not clear, after which the rate of absorption became steady and constant over the interval measured.

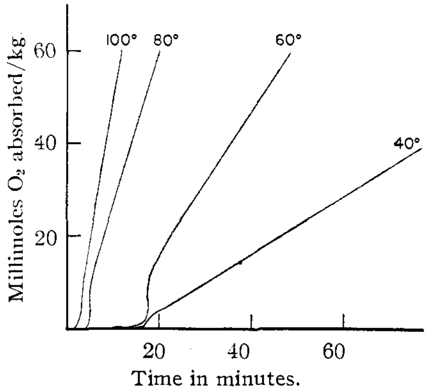

Fig. 2.—Typical oxygen absorption curves at 40, 60 80 and 100°.

The rate of oxygen absorption increased approximately two-fold with each 20° rise in temperature. This temperature coefficient has no appreciable significance since it is dependent on a number of arbitrary conditions associated with the oxygen absorption measurements, including such factors as the rate of shaking, the shape of the flask, and the rate of diffusion of oxygen into the samples.

The measured peroxide values have been plotted in relation to oxygen absorbed in Fig. 3. Each curve represents one temperature and is based on 7 to 15 well distributed points. A correction has been made for a lag in the manometer readings which is apparently due to a fall in the concentration of dissolved oxygen after the reaction has begun. The curves are virtually straight lines up to peroxide values of 300 m. e./kg. The average deviation of the experimental points from these curves is about 3%. These deviations

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(12) Holman, Lundberg, Lauer and Burr, THIS JOURNAL, **67**, 1285 (1945).

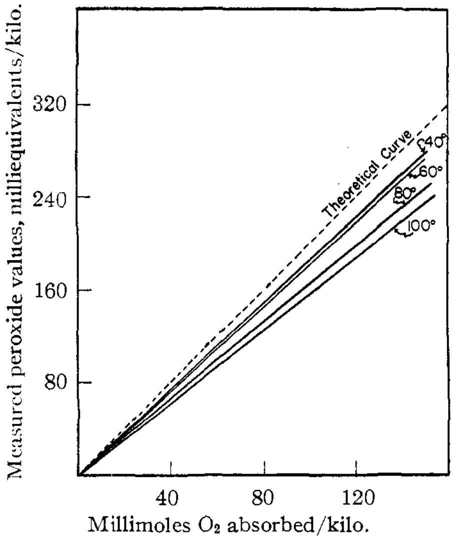

Fig. 3.—Peroxide accumulation in relation to oxygen absorption at 40, 60, 80 and 100°.

may be attributed to errors in the oxygen absorption measurements rather than in the peroxide determinations. This conclusion is based on the fact that the deviations of experimental points from the curves drawn in Fig. 4, where the spectral absorptions at two wave lengths are plotted in relation to peroxide values, are considerably smaller. In spite of such experimental errors in the oxygen absorption measurements, it is apparent that there are differences between the measured and theoretical maximum peroxide values, and that the differences increase with increasing temperature. In Fig. 4 it is shown that in the wave length region corresponding to diene absorption, *i. e.*, at 2325 Å., the absorption varies in direct proportion to the peroxide value, and this relationship is entirely independent of temperature in the range from 40 to 100°. The broken line in the figure accurately represents the data obtained at all temperatures. The average deviation of all points obtained at all temperatures from this straight line is approximately 1%. The constancy of this relationship indicates that, even if several spectroscopically differentiated types of peroxides are formed, the same types are formed in the same proportions at all temperatures between 40 and 100°. The considerable differences in types of peroxides that have been reported to be formed in oxidizing fats at various temperatures must therefore be regarded primarily as differences in the types of peroxides of oleic acid or of fatty acids other than linoleic acid.

The constancy of this relationship further indicates that all of the conjugated dienes are present as peroxides. Not all of the peroxides, however, are present as conjugated dienes. The presence of stable peroxides having no diene conjugation appears to be unquestionable, such peroxides having been isolated by Bergström.[7]

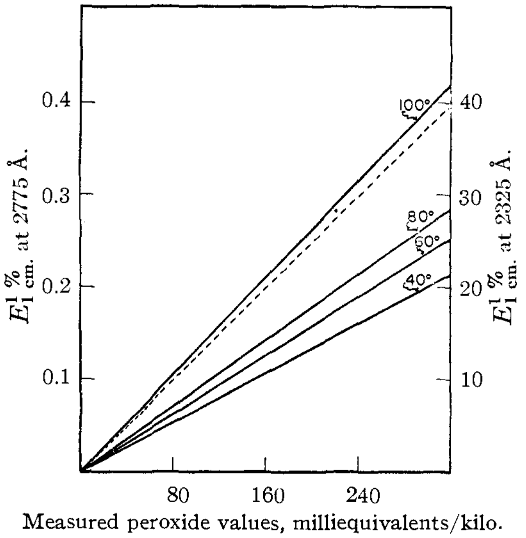

Fig. 4.—$E^{1\%}_{1\text{ cm.}}$ in relation to peroxide accumulation at 40, 60, 80 and 100°; ----, 2325 Å.; ——, 2775 Å.

Assuming that the molecular absorption coefficients of the conjugated linoleate peroxides and of the corresponding conjugated linoleates are identical (actually, slight differences would be expected), it may be calculated from the known coefficients for the latter that approximately 70% of the peroxides are present as conjugated dienes. On the basis of a similar assumption, the same ratio was obtained by Bolland and Koch at 37°.[5] According to them, if one applies the concept of a resonating free radical in this case, this ratio indicates that there is an approximately even distribution of linoleate free radicals between three resonance structures. The constancy of the ratio with respect to temperature is in accord with this concept.

Because of the constancy of the proportion of conjugated diene peroxides in Fig. 4, the differences between the experimental and theoretical peroxide values in Fig. 3 cannot be attributed, except in a minor degree, to a thermal decomposition of the stable peroxides except in the improbable events that the conjugated diene peroxides and the spectrally non-absorbing peroxides are decomposed in the same ratio in which they are formed, and that the conjugated diene peroxides in decomposing lose their diene conjugation. It has been previously shown in an oxidized natural fat containing conjugated diene peroxides that the spectral absorption in the region concerned increased when the peroxides were decomposed by superheated steam.[12,13]

At 2775 Å. the relationships between spectral absorption and peroxide value are also direct proportions but in these cases the absorption increases with increasing temperature, as is indicated by successively greater slopes of the solid straight lines at higher temperatures. The mag-

(13) Lundberg, Holman and Burr, *Oil & Soap*, **23**, 10 (1946).

nitude of the absorption suggests, on first examination, that these changes constitute only a very small part of the total reaction. However, the addition of alkali immediately increases the absorption by approximately 6 to 8 times, as is shown by a comparison of the ratios of absorptions to peroxide values in columns 2 and 3 of Table I.

TABLE I

RATIOS OF $E^{1\%}_{1\,\text{cm.}}$ TO MEASURED PEROXIDE VALUES AT 2775 Å.

Temp., °C.	Alcohol	KOH 15 min.	KOH 48 hr.
40	0.00067	0.0056	0.0212
60	.00079	.0065	.0177
80	.00089	.0069	.0163
100	.00131	.0077	.0166

It is evident therefore that secondary products, apparently consisting of diene ketones that possess either linear conjugation or cross conjugation, are formed in appreciable amounts. These are presumably enolized by alkali into conjugated triene structures.

The magnitude of the absorption at 2775 Å. suggests, though the evidence is not conclusive, that these secondary products may be in a large measure formed by action of the oxygen that is represented in the differences between the experimental and theoretical peroxide values in Fig. 3. There does not appear to be sufficient evidence available to define the reaction or reactions by which these chromophores are formed, although several possibilities are evident. The constancy of the proportion of conjugated diene peroxides irrespective of temperature, the straight line relationships between spectral absorption at 2775 Å. and peroxide values, and the dependence of the latter relationships on temperature all taken together suggest that the chromophores absorbing at 2775 Å. are not formed from the stable peroxides either by decomposition or by subsequent oxidation, but that they are formed in reactions that are concurrent with the formation of the stable peroxides.

A comparison of the figures in columns 2 and 3 also suggests that not only the amount but the chemical nature of these lesser products is to some extent dependent on the temperature of the autoxidation. Thus, even in its earliest stages, the autoxidation of methyl linoleate appears to be a complicated mixture of reactions, among which the predominant reaction is the formation of stable peroxides by attack on the methylene carbon between the double bonds.

Summary

A study of the early stages of the autoxidation of pure methyl linoleate at 40, 60, 80 and 100° has been made.

Most but not all of the absorbed oxygen may be found in the form of relatively stable linoleate peroxides. A small fraction of the absorbed oxygen at any given temperature is not found in the peroxides, but this fraction increases with increasing temperature.

At all levels of oxidation up to 300 m. e./kg. of peroxide and at all temperatures between 40 and 100°, a constant fraction of the total peroxides is present as conjugated dienes; all of the conjugated dienes are present as peroxides.

In addition, secondary products showing absorption at 2775 Å. are formed in proportion to the oxygen uptake. The proportion of these chromophores increases with increasing temperature. Changes in absorption upon the addition of alkali suggests that they are largely ketonic in character, and that their character is to some extent dependent on the temperature of autoxidation. They appear not to be formed from the stable peroxides but by reactions concurrent with the formation of stable peroxides.

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The Ammonolysis of Ethyl Iodide by Liquid Ammonia[1, 1a]

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The rate of reaction between ethyl iodide and liquid ammonia and the nature of the reaction products have been the subject of conflicting reports in the chemical literature. White, Morrison, and Anderson[2] reported that the reaction is very slow at −33°, but they did not isolate or identify any of the reaction products. Picon[3] stated that the reaction was complete in twenty-four hours at "ordinary temperatures," and after four hours yielded 28% $C_2H_5NH_2$, nearly an equal quantity of $(C_2H_5)_2NH$ and a very small quantity of $(C_2H_5)_3N$. Markova and Shatenshtein[4] considered the reaction at 25° to be sufficiently slow to permit rate measurements and reported primary amine as the only reaction product. None of these studies has accounted for more than 50% of the ethyl iodide employed in the reactions and all leave the impression that the reaction is slow.

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(1) This work was supported, in part, by grants from the University Research Institute, Project No. 25.

(1a) Presented at the Southwestern Regional Meeting of the American Chemical Society, Dallas, Texas, December 13, 1946.

(2) White, Morrison and Anderson, THIS JOURNAL, **46**, 963 (1924).

(3) Picon, *Bull. soc. chim.*, [4] **35**, 979 (1924).

(4) Markova and Shatenshtein, *Compt. rend. acad. sci. U. R. S.S.*, **35**, 68–70 (1942).