Spectrophotometric Studies of the Oxidation of Fats. VI. Oxygen Absorption and Chromophore Production in Fatty Esters[1]

By Ralph T. Holman and George O. Burr

Investigations concerning the changes in ultra-violet absorption spectra of oxidizing fats and fatty acids and esters have shown that oxidation of the unsaturated components of fats lead to the development of new absorption maxima.[2-5] In the qualitative studies reported earlier from this Laboratory[5] the degree of oxidation was followed by time of exposure to air and by peroxide value. In the present investigation, systematic studies of the relationship of oxygen absorption to absorption band development have been made with the esters of the common unsaturated fatty esters.

In order that the conditions be as mild as possible and still have oxidation proceed rapidly enough to measure conveniently, and in order that results of these investigations may be comparable to similar studies of the enzymatic oxidation of fats,[6] the experiments were conducted at 37°, a temperature considerably lower than that usually used in fat autoxidation studies.

Experimental

The physical properties of the ethyl oleate, ethyl linolate and ethyl linolenate used in this study have been reported elsewhere.[5] The methyl arachidonate was prepared from fresh hog's liver fat through the octabromides (m. p. 225°) and had an iodine number of 316.5 (theory 319).

The oxidations were carried out on approximately 50-mg. samples of the pure esters in small Warburg respirometer vessels held at 37°. Several samples of an ester were run simultaneously, one at a time being removed when its desired oxygen uptake had been attained. Three ml. of alcohol, distilled from potassium hydroxide, was added to each of the oxidized samples, the ester dissolved completely, and an aliquot part diluted for spectrophotometric measurement with the Beckman spectrophotometer. When the measurements in alcohol were completed, the alcoholic solutions were diluted with equal volumes of 20% aqueous potassium hydroxide to observe the effect of alkali upon the spectrum.

Results and Discussion

The oxidation of ethyl oleate proceeded so slowly that no appreciable change in spectrum was noted during the experiment. The rate of oxygen uptake was found to increase with the number of double bonds in the fatty acid, as is common knowledge.[7] This was also noted in the rates of development of the absorption maxima in both alcoholic and alkaline solution (Figs. 1–4). As the absorption spectra were followed through the course of the oxidations of the linolate and linolenate it was noted that the increases in absorption over that of the fresh material were somewhat greater than those reported for the same samples of esters oxidized at a higher temperature.[5] In the case of linolenate definite fine structure was apparent in both alcoholic and alkaline solutions of the samples oxidized at 37° (Figs. 5, 6, 7) but was lacking in the samples oxidized at higher temperatures.[5] From these observations it would appear that at higher temperatures the strongly absorptive primary oxidation products are decomposed, and that those products of linolenate oxidation which exhibit fine structure in their spectra are converted to compounds having broad absorption bands in the same region but lacking the fine structure. The decomposition of a conjugated hydroperoxides such as suggested by Bolland and Koch[8] to conjugated ketones could account for such a change.

The spectra of oxidized arachidonate samples appear qualitatively the same as the oxidized linolenate spectra. However, the evidence for fine structure is somewhat obscured, and the increased absorption in the longer wave lengths in alkaline solution is considerably greater than in the corresponding spectra of oxidized linolenates. The lack of pronounced fine structure may be due to greater lability of the more highly unsaturated intermediate oxidation products, and at 37° these may behave somewhat as the products of linolenate oxidation do at higher temperatures.

A comparison of the visible color ($E_{1\text{ cm.}}^{1\%}$ at 4000 Å.) produced by alkali upon samples of linolate, linolenate, and arachidonate which have absorbed about 1 mole of oxygen (Figs. 5, 6, 7) bears out the observation made in qualitative studies[9] that the depth of the color of oxidized fatty acids in alkali is greatly affected by the degree of unsaturation of the acid. In the present study the extinctions at 4000 Å. of linolate, linolenate and arachidonate, each containing about 1 mole of oxygen/mole ester, are about 1, 6

(1) This work was supported by grants from the Hormel Research Foundation, the National Livestock and Meat Board, and the National Dairy Council.

(2) J. H. Mitchell and H. R. Kraybill, THIS JOURNAL, **64**, 988 (1942).

(3) E. H. Farmer, H. P. Koch and D. A. Sutton, *J. Chem. Soc.*, **122**, 541 (1943).

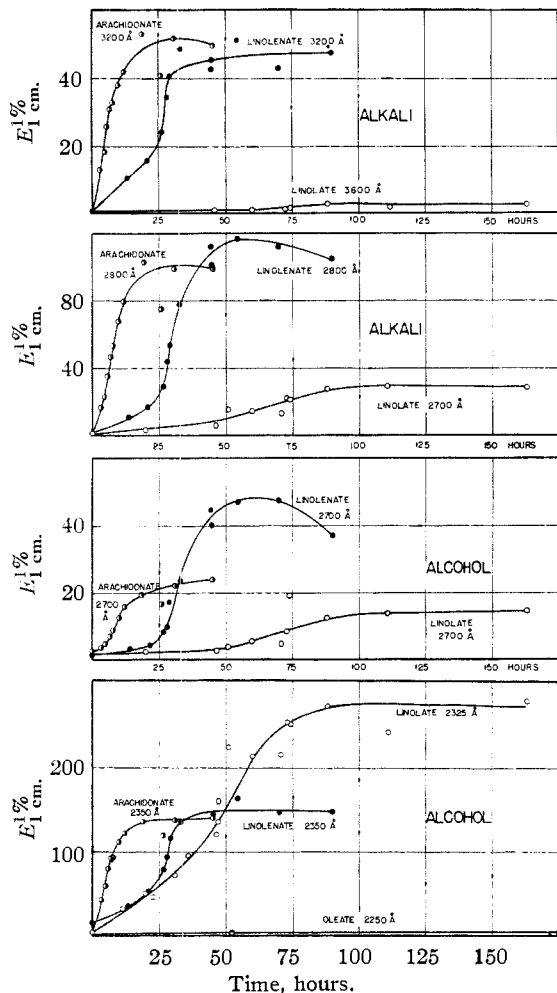(4) L. J. Filer, K. F. Mattil and H. E. Longenecker, *Oil & Soap*, **22**, 196 (1945).

(5) R. T. Holman, W. O. Lundberg, W. M. Lauer and G. O. Burr, THIS JOURNAL, **67**, 1285, 1386, 1390 (1945).

(6) R. T. Holman and G. O. Burr, *Arch. Biochem.*, **7**, 47 (1945).

(7) A. J. Stirton, J. Turer and R. W. Riemenschneider, *Oil & Soap*, **22**, 81 (1945).

(8) J. L. Bolland and H. P. Koch, *J. Chem. Soc.*, 445 (1945).

(9) R. T. Holman, W. O. Lundberg and G. O. Burr, THIS JOURNAL, **67**, 1669 (1945).

Figs. 1–4.—Development of chromophores during the oxidation of fatty esters.

and 20, respectively (top curves in Figs. 5, 6 and 7). Hence it may be considered that the in-

TABLE I

FORMATION OF CHROMOPHORES IN AUTOXIDIZED ESTERS

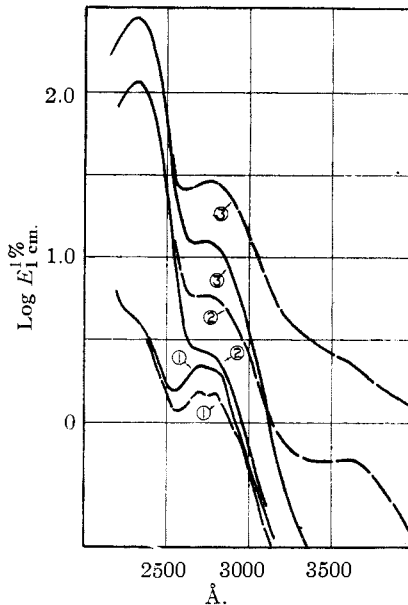|		$E_{1\ cm.}^{1\%}$/mole oxygen		"Monohydroperoxide" calculated from O_2 content	
		At 1 mole			At 1 mole
			oxy-		oxy-
Ester	Å.	Early stages	gen/mole ester	Early stages	gen/mole ester
Ethyl	2325	601	260	18,500	8020
lino-	2700	5.4	15	167	462
late	2700 (KOH)	26.8	32	....	....
	3600 (KOH)	1.5	2.5	....	....
Ethyl	2350	335	157	10,260	4810
lino-	2700	28	45	857	1380
lenate	2800 (KOH)	140	115	....	....
	3200 (KOH)	104	50	....	....
Methyl	2350	219	140	6960	4450
arachi-	2700	15.4	22	490	700
donate	2800 (KOH)	108	102	....	....
	3200 (KOH)	78.2	55	....	....

Fig. 5.—Ultraviolet absorption spectrum of ethyl linolate oxidized at 37°: 1, fresh ester; 2, 0.165 mole oxygen/mole ester; 3, 0.898 mole oxygen/mole ester; — in alcohol; – – – in potassium hydroxide.
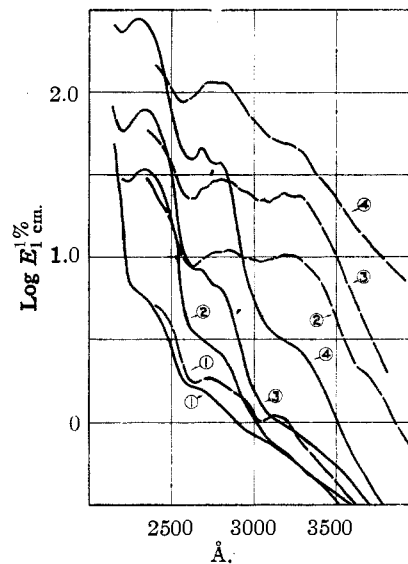

Fig. 6.—Ultraviolet absorption spectrum of ethyl linolenate oxidized at 37°: 1, fresh ester; 2, 0.064 mole oxygen/mole ester; 3, 0.219 mole oxygen/mole ester; 4, 0.97 mole oxygen/mole ester; — in alcohol; – – – in potassium hydroxide.

tensity of alkali color at this wave length has no quantitative meaning unless the fatty acids are known. Since all of the ultraviolet bands likewise have different intensities per mole of oxygen uptake for each fatty acid, the value obtained with mixed fatty acids will depend upon the composition of the mixture (Table I).
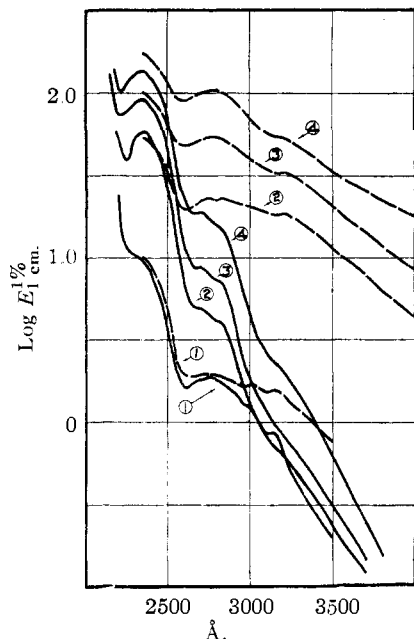
The development of the chromophores in

Fig. 7.—Ultraviolet absorption spectrum of methyl arachidonate oxidized at 37°: 1, fresh ester; 2, 0.227 mole oxygen/mole ester; 3, 0.419 mole oxygen/mole ester; 4, 0.995 mole oxygen/mole ester; — in alcohol; - - - in potassium hydroxide.

oxidizing esters show the characteristic curves of autocatalytic reactions. The more unsaturated the ester, the shorter is the induction period as measured by any one of the four major chromophores produced upon oxidation (Figs. 1–4). The maximum rate of chromophore formation in the linolate is never as great as it is in arachidonate and linolenate.

During the early phases of oxidations, the development of most of the chromophores is proportional to the oxygen uptake, but in the latter phases, particularly after 0.9 mole of oxygen per mole ester have been absorbed, the relationship between chromophore content and apparent oxygen content becomes very irregular (Figs. 8, 9, 10). This irregularity is probably related to the production of volatile products of oxidation during the latter stages, the appearance of which counterbalance the disappearance of oxygen from the system. These secondary decompositions become so rapid that in a few cases the apparent oxygen uptake reached a maximum and then decreased with time near the end of the experiments.

From the comparisons of the development of the "diene" and "triene" and the alkaline "triene" and "tetraene" chromophores, it will be seen that the greatest increase in each of these portions of the spectrum upon oxidation of the esters is attained by that ester which originally has the appropriate number of double bonds which upon conjugation would absorb in that region. Thus, in alcoholic solutions oxidized linolate attains the greatest absorption maximum
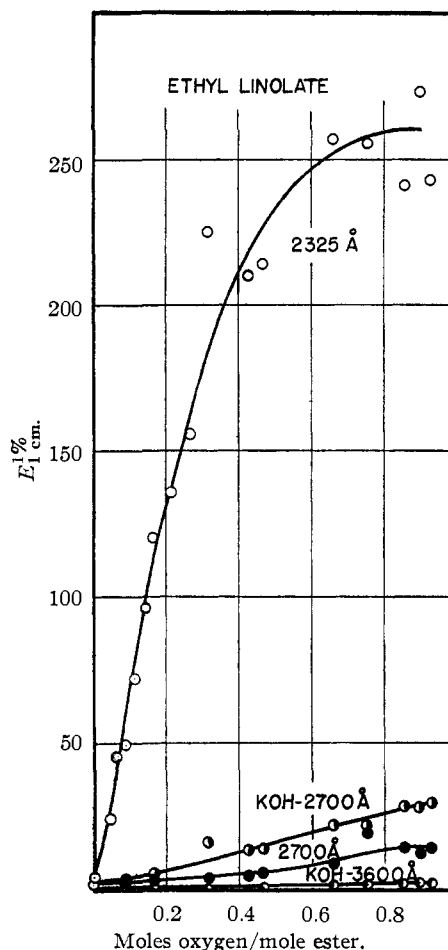

Fig. 8.—Relationship between chromophore formation and oxygen absorption in oxidizing fatty esters.

in the 2300 Å. region, linolenate attains the greatest absorption in the 2700–2800 Å. range in alcohol and in alkali, and arachidonate attains the highest absorption in the 3200 Å. region in alkaline solution (Figs. 1–4). From this it appears that although all three of the esters form chromophores in these regions of the spectrum, the ester containing the correct number of double bonds for potential conjugation most readily forms the chromophores absorbing in a given portion of the spectrum.

It will also be seen that the 2300 Å. band in alcoholic solution in all cases reaches higher extinction values than any other chromophore (Figs. 8, 9, 10). Thus even in methyl arachidonate having four double bonds, the conjugated two double bond chromophore reaches the highest absorption. This probably does not mean that the chromophores absorbing in the longer wave lengths are less absorptive but that they are present in lower concentrations.

Bolland and Koch[8] recently reported observations that in the early stages of autoxidation of ethyl linolate (less than 0.36 mole oxygen/mole

Fig. 9.—Relationship between chromophore formation and oxygen absorption in oxidizing fatty esters.
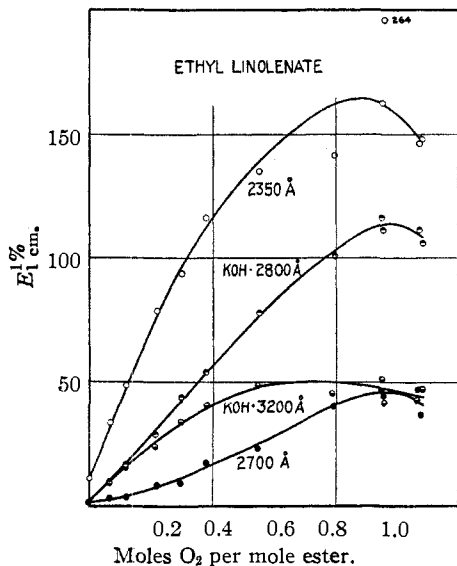

Fig. 10.—Relationship between chromophore formation and oxygen absorption in oxidizing fatty esters.

ester) peroxides accounted for all of the oxygen uptake, and that the product had the characteristics corresponding to a hydroperoxide which was about 70% conjugated. The molecular extinction coefficient observed for their product at 2315 Å. was 22700. In the present study during the initial stages of the oxidation of ethyl linolate the chromophore absorbing at 2325 Å. was found to have an average $E^{1\%}_{1\,cm.}$ of 601 per mole of oxygen absorbed (Table I). If it is assumed that this chromophore is the ethyl linolate hydroperoxide, the molecular extinction coefficient becomes 18500, which is 58% of the maximum value (32000) reported by Kass[10] for conjugated octadecadienoic acids. The range of values obtained is given in Table II. It will be seen that there were several samples which reached a molecular extinction coefficient equal to about 66% of the theoretical maximum. Before strict comparisons

TABLE II

"DIENE" CHROMOPHORE DEVELOPMENT IN EARLY STAGES OF LINOLATE OXIDATION

O₂ uptake moles/mole ester	Increased $E^{1\%}_{1\,cm.}$ at 2325 Å.	$E^{1\%}_{1\,cm.}$/mole oxygen	ϵ
0.044	20.2	460	14,200
.064	41.9	655	20,200
.092	45.4	493	15,200
.116	68.2	588	18,100
.145	92.3	634	19,600
.165	115.9	702	21,600
.217	131.9	608	18,750
.267	151.9	568	17,500
.316	221.9	702	21,600
	Av. 601	Av.	18,500

can be made between the present data and the primary standard of conjugated material, it is necessary that the extinction coefficient of such a standard be determined with our instrument. However, the present results are in rather good agreement with the 70% conjugation reported by Bolland and Koch (also based on a possible extinction coefficient of 32000). These authors point out that it is likely that their value of 22700 is due to incomplete conjugation rather than to a lower extinction coefficient for conjugated peroxidolinolate. They argue that if the hydroperoxide group were distributed at random on the three most likely carbons (9, 11, 13) there would result 67% conjugated diene monohydroperoxide. The 11-hydroperoxide would be unconjugated, whereas the 9 and 13 compounds would be conjugated.

If a similar argument were applied to the more complicated cases of linolenic and arachidonic acids the points of attack on the postulated free radicals would be at the 9, 11, 14 and 16 carbons and the 8, 10, 13, 16 and 18 carbons, respectively. If no multiple oxidation of a given fatty acid took place so that only diene conjugation resulted, the predicted maximum values for random distribution would be 50% conjugation (ϵ = 16,000) for linolenic acid and 40% (12,800) for arachidonic acid.[11] The 2350 Å. values found (Table I) are considerably lower than the above. This deviation would be predicted from the curves in Figs. 1–10. It is clear that only linolate produces a high diene absorption without an appreciable rise in the 2700 band or in secondary oxidation products which produce bands in alkali. In both linolenate and arachidonate the increasing intensities of the other bands follow closely upon that at 2350 Å. Oxygen-containing compounds isomerizable with alkali accumulate rapidly and without appreciable induction period in the arachidonate. There is very little uncomplicated

(10) J. P. Kass, "Protective and Decorative Coatings," edited by J. Mattiello, John Wiley and Sons, New York, N. Y., 1944, p. 394.
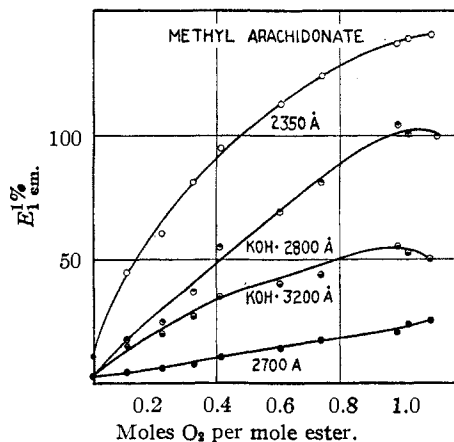
(11) This estimate is based on the assumption that the carbons adjacent to active methylene groups are not points of attack.

triene conjugation in linolenate as shown by the long induction period in the rise of the 2700 band (Fig. 3) and the much more rapid increase of the oxygen compounds sensitive to alkali (Figs. 1, 2 and 9). From this it may be concluded that there is little evidence in these data that a 9,16-dihydroperoxide is formed in more than traces from linolenate. That monohydroperoxides are the chief primary oxidation products of the linolenate and arachidonate is indicated by the fact that the maximum chromophore intensity is at 2350 Å.

These speculations at first seem to contradict earlier findings that peroxides were not responsible for the absorption band near 2300 Å.[5] It should be pointed out that extinction at this wave length is proportional to peroxide content only in the early stages of oxidation or when the oxidizing conditions are not conducive to peroxide destruction. Destruction of the peroxides leads to compounds which still absorb strongly at 2300 Å. These observations do not refute the contention that the conjugation observed in oxidized fats is formed through the conjugated hydroperoxide.

After this paper went to press the authors read a contribution by Sune Bergström in which similar results were reported.[12]

Summary

1. The rates of development of the various chromophores appearing during the oxidation of ethyl linolate, ethyl linolenate and methyl arachidonate have been followed and related to the oxygen absorbed.

2. At 37° oxidation of linolenate, and possibly arachidonate, is accompanied by the formation of chromophores which exhibit fine structure in the spectra of their alcoholic and alkaline solutions. This was not observed with ethyl linolate.

3. The rate of formation of chromophores absorbing at 2325 Å. in oxidizing linolate and the rate of oxygen uptake agrees with the postulated formation of a conjugated monohydroperoxide as suggested by Bolland and Koch.

4. The development of color or of ultraviolet chromophores cannot be taken as a measure of oxygen uptake (degree of oxidation) unless the composition of the fatty acid mixture is known.

(12) Bergström, *Arkiv för Kemi, Mineralogi och Geologi*, **21A**, No. 14 (1945).

### Absorption Spectra. III. Cyclic β-Diketones

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Continuing our studies on the relationship between structure and absorption spectra of molecules,[1] we have investigated the spectra of several simple β-diketones and the effect of bromine substitution upon the fundamental diketonic spectrum. Although previous investigators[2,3] have mentioned the influence of bromine atoms adjacent to carbonyl groups upon absorption, no extensive study of this phenomenon has been reported.

The acidic nature of cyclic β-diketones is ascribable to the fact that upon enolization of one of the hydrogen atoms between the carbonyl groups a grouping is formed which has all the properties of, and is vinylogous with, a carboxylic acid. This acidic form (Ib) of a typical cyclic β-diketone, 5,5-dimethylcyclohexanedione-1,3, is one of the possible formulas (I) which may be written for such a compound.

I
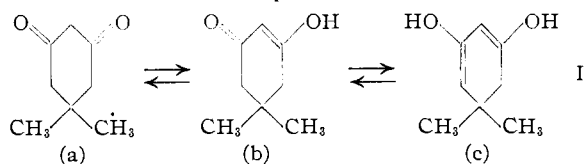
The present work shows that form Ib, with contributions of forms such as those shown in II

II
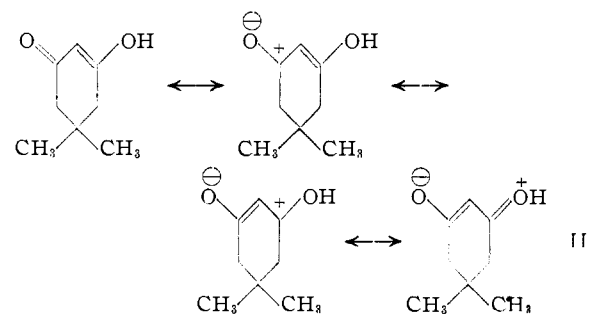
and their dissociated or ionic forms, III

III
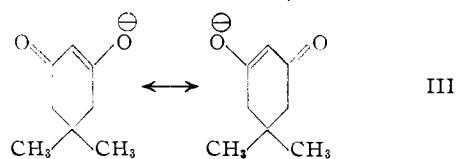
are responsible for many of the light absorptive properties associated with such compounds.

In contrast to isolated carbonyl groups which absorb only very weakly in the ultraviolet (log ε 1 → 2, λ max. 270–300 mμ), β-diketones are characterized by intense absorption.[2,4,5] The

(1) Blout and Gofstein, THIS JOURNAL, **67**, 13 (1945); Blout and Eager. *ibid.*, **67**, 1315 (1945).

(2) Graham and Macbeth, *J. Chem. Soc.*, **121**, 2601 (1922).

(3) Woodward, THIS JOURNAL, **63**, 1123 (1941).

(4) Grossmann, *Z. physik. Chem.*, **109**, 305 (1924).

(5) Woodward and Blout. THIS JOURNAL, **65**, 562 (1943).