

An examination of Figs. 5 and 6 shows that the absorption spectra of these substances are not much like those of oxidized linoleic acid. Special attention should be called to phorone and crotylideneacetone. The former has the structure postulated by Scheiber³ for an oxidation product of linoleic acid. Only in alkaline solution does oxidized linoleic acid exhibit broad bands at 2700 and 3700 Å. much like those of phorone but of different relative intensity. The bands of phorone and crotylideneacetone are so intense that a fraction of one per cent. could account for all of the absorption at 2700 Å. shown by the most highly oxidized linoleic acid used in this study.

Summary

1. The absorption spectra of ethyl linolate, highly purified linoleic acid, and 10,12-linoleic acid were studied before and after oxidation in neutral and alkaline alcoholic solutions.

2. Autoxidation of each of these compounds is accompanied by an increase in the absorption at 2750 Å. which is not directly related to peroxide value.

3. Oxidation of conjugated linoleic acid greatly reduces the absorption at 2300 Å.

4. Cool alkali has no effect upon the absorption of the conjugated or unconjugated fresh fatty acids but produces marked changes in the oxidized products.

5. Absorption spectra of acrolein, α -methylacrolein, mesityl oxide, phorone, crotylideneacetone, and β -ionone in alcohol and alkali are shown as examples of unsaturated conjugated carbonyl compounds.

6. The autoxidation products of linoleic acid are spectroscopically unlike those of oleic acid.

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Spectrophotometric Studies of the Oxidation of Fats. III. Ultraviolet Absorption Spectra of Oxidized Octadecatrienoic Acids

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In previous investigations^{3,4} the changes of ultraviolet absorption spectra were followed during the courses of oxidation of fatty acids containing one or two double bonds. The present report presents the changes in spectrum induced by the oxidation of the fatty acids containing three double bonds, isolated or conjugated.

Experimental

The ultraviolet absorption spectra were determined using the Beckman quartz spectrophotometer. 95% ethyl alcohol distilled over potassium hydroxide was used as solvent. After the spectra in alcoholic solutions were determined, 5.0 cc. of the alcoholic solutions were diluted with 5.0 cc. of 20% aqueous potassium hydroxide, and the spectra in alkali determined immediately.

Linolenic acid and ethyl linolenate were exposed to air in an oven at 63°, the temperature used in an oven stability test.⁵ A second sample of linolenic acid which had

accidentally become unsealed in the cold room (-20°) was also included in this study. The exact length of time of exposure to air is not known, but it was of several months duration. Elaidolinolenic acid was exposed to air at 78°. The temperature at which pseudoeleostearic acid was exposed to air was elevated to 77°, in order that the fatty acid might be in the liquid state. It was found that the oxidation was so rapid that insoluble films formed within a day, so when α -eleostearic acid was oxidized the temperature was reduced to 48°, just above the melting point of the sample. The temperature at which β -licanic acid was oxidized was elevated to 97° to insure that the fatty acid was in the liquid state. Again it was observed that films formed very rapidly. Peroxide values were determined by a modification of Wheeler's method.⁶ It was observed in earlier work that peroxide values showed no correlation with the spectral changes, and inasmuch as peroxide values are a poor measure of the degree of oxidation, they were discontinued in these studies which are to be extended, using oxygen absorption as an index of degree of oxidation.

The linolenic acid used in this study was prepared by low temperature crystallization and was kindly supplied by Dr. J. B. Brown. Ethyl linolenate, I. V. = 247.5, was prepared by debromination of the hexabromides. Elaidolinolenic acid, m. p. 27-28°, was prepared by Dr. J. P. Kass. Pseudoeleostearic acid, m. p. 77.0-77.5°, was prepared by the method of Kass and Burr.⁷ α -Eleostearic acid was prepared from tung oil and had a m. p. of 45°. β -Licanic acid, m. p. 92-96°, was prepared from oiticica oil kindly supplied by Brazil Oiticica Co. of New York.

(1) This paper is taken in part from a thesis presented by Ralph T. Holman to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree, June, 1944. The work was aided by grants from the Hormel Research Foundation, the National Live Stock and Meat Board, and the National Dairy Council.

(2) Present address: Hormel Institute, Austin, Minnesota.

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

(4) R. T. Holman, W. O. Lundberg and G. O. Burr, *ibid.*, **67**, 1386 (1945).

(5) W. O. Lundberg, H. O. Halvorson and G. O. Burr, *Oil and Soap*, **21**, 33 (1944).

(6) A. E. King, H. E. Roschen and W. H. Irwin, *ibid.*, **10**, 105 (1933).

(7) J. P. Kass and G. O. Burr, *THIS JOURNAL*, **61**, 3292 (1939).

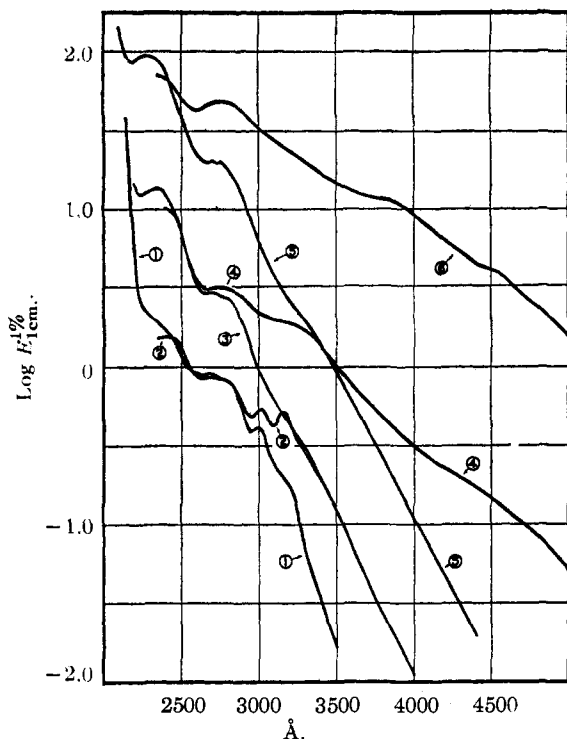


Fig. 1.—Ultraviolet absorption of linolenic acid: (1), fresh linolenic acid; (2), (1) in KOH; (3), (1) in air at -20° for several months, P. V. = 60; (4), (3) in KOH; (5), (1) in air at 63° 27 hr., P. V. = 490; (6), (5) in KOH.

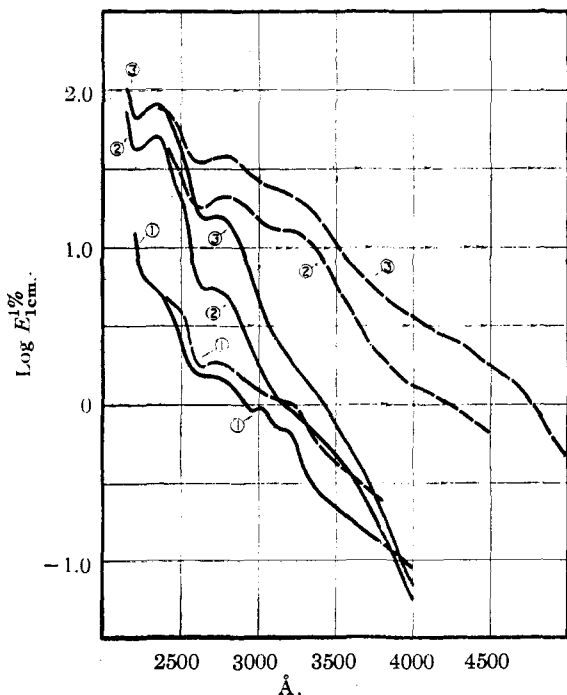


Fig. 2.—Ultraviolet absorption of ethyl linolenate: (1), ethyl linolenate; (2), (1) exposed to air 60 hours at 63° ; (3), (1) exposed to air 138 hours at 63° ; ——— in alcoholic solution, - - - - - in KOH.

We are indebted to Mr. David Molander and Mr. Norman Kretschmer for aid in the preparation of ethyl linolenate and pseudooleostearic acid, respectively.

Results and Discussion

Accompanying the autoxidation of linolenic acid is an increased general absorption and the rise of absorption maxima at 2750 and 2350 Å. (Fig. 1). The addition of alkali to the oxidized fatty acid increases markedly the absorption in the longer wave lengths and also elevates the maximum at 2750 Å. The same changes were found in oxidized ethyl linolenate (Fig. 2) and the oxidized *trans* isomer of linolenic acid (Fig. 3).

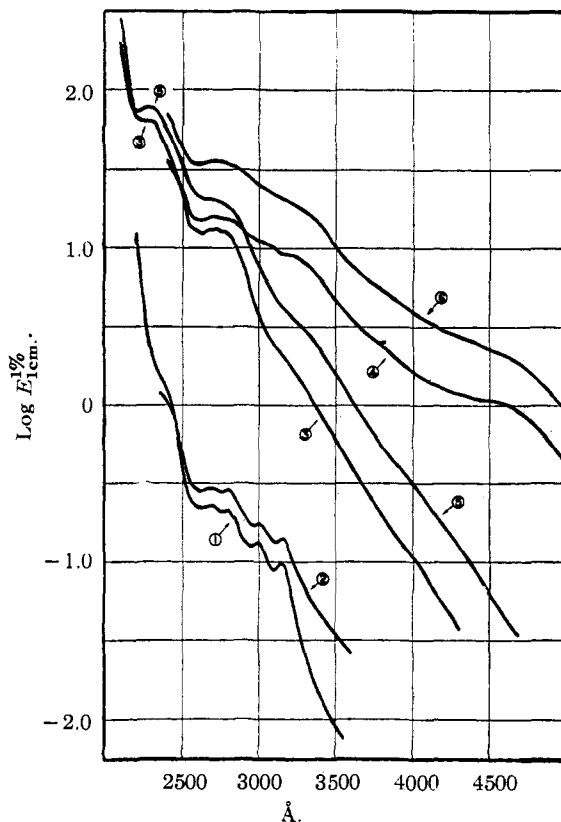


Fig. 3.—Ultraviolet absorption of elaidolinolenic acid: (1), fresh elaidolinolenic acid; (2), (1) in KOH; (3), (1) in air 78° for 27 hours, P. V. = 26; (4), (3) in KOH; (5), (1) in air 78° for 106 hrs., P. V. = 35; (6), (5) in KOH.

The spectral curves are very similar to those of oxidized oleates³ and linoleates.⁴ In Fig. 7 are comparable curves of typical samples of oleic, linoleic and linolenic acids autoxidized under similar conditions to high peroxide values. In alcohol all have bands at 2750 Å. which are intensified by alkali and which do not show fine structure. Both of these properties indicate that the absorption at 2750 Å. is due to oxygen-containing chromophores and not to simple conjugated trienes, whose bands possess fine structure and are unaffected by alkali (Figs. 4, 5, 6). The fine structure noted in the spectra of the

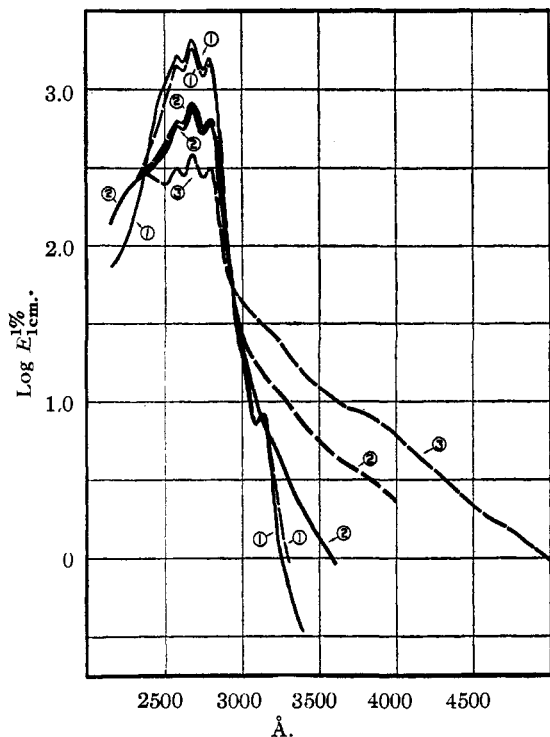


Fig. 6.—Ultraviolet absorption of β -licanic acid: (1), β -licanic acid; (2), (1) in air at 97° for 9 hours; (3), (1) in air at 97° for 24 hours; ——— in alcoholic solution, — · — · — in KOH.

The reactions leading to compound IV could occur in autoxidation of fats. Oxido compounds have been demonstrated among products of fat oxidation.¹⁰ Upon exposure of oxidostearic acid to warm air decomposition products are formed which show increased absorption in the region of 2750 Å. and which respond to alkali by increasing the absorption markedly to form a maximum near 2800 Å. Formation of carbonyl compounds from oxido compounds could account for smooth maxima in oxidized fats and the sensitivity of the products to alkali. Subsequent bleaching could then give rise to a triene conjugated system. These speculations are in agreement with the observations that oxidation of non-conjugated acids produces smooth bands at 2750 and 2350 Å., whereas bleaching after oxidation produces bands showing fine structure characteristic of the conjugated fatty acid containing one more double bond than the parent non-conjugated fatty acid.

If the band at 2750 Å. in oxidized linoleates were due to formation of conjugated trienes, some mechanism such as that involved in bleaching must also occur in oxidation. If this be the case, then oxidation of linolenates should give rise to conjugated tetraenes. Thus far no band at 3000–3100 Å. has been observed in oxidized linolenates or in other oxidized fatty acids. On the contrary, it has been noted that upon oxidation

(10) H. A. Mattill, *Oil and Soap*, **18**, 73 (1941).

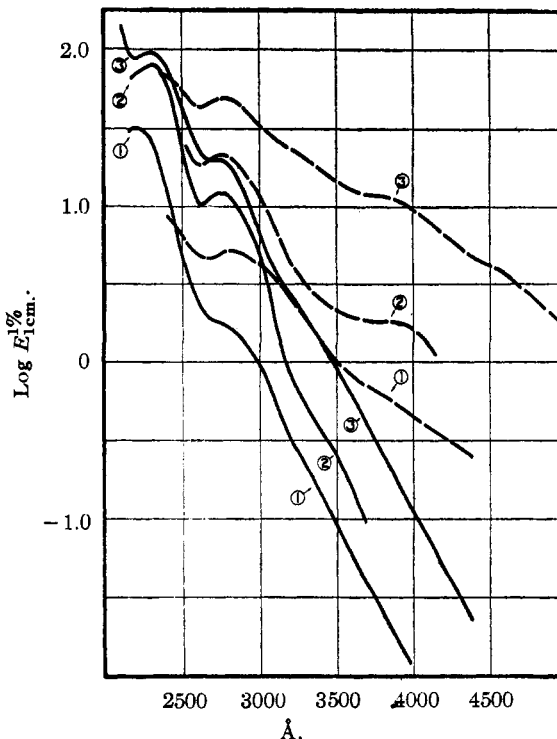


Fig. 7.—Ultraviolet spectra of oxidized unsaturated acid: (1) oleic acid in air 63° for 40 days, P. V. = 55.0; (2) linoleic acid in air at 63° for 96 hours, P. V. = 280; (3) linolenic acid in air at 63° 27 hours, P. V. = 490; ——— in alcohol, — · — · — in KOH.

of α -eleostearic acid, pseudoeleostearic acid, and β -licanic acid the band at 3100 Å. due to traces of conjugated tetraene disappears in the first stages of the oxidation. If then any conjugated tetraene were formed from unconjugated material it could exist only momentarily. Likewise, if any conjugated triene were formed by oxidation of non-conjugated material, it would exist only momentarily because it is more rapidly oxidized than are non-conjugated systems.¹¹

As shown by Brauer and Steadman¹² in their study on the oxidation of β -eleostearic acid, the oxidation of the conjugated systems results in a decreased absorption in the range of 2600–2800 Å. This is accompanied by an increased absorption both in the region of 2300 Å. and at wave lengths longer than 3200 Å. The increased absorption at 2300 Å. may be due to degradation of the triene system to dienes. The increased absorption at the longer wave lengths imparts color to the oxidized acid, but the increase in color is not due to the development of an absorption maximum below 4000 Å. It is interesting to note that oxidation of conjugated triene did not lead to the formation of conjugated tetraene.

(11) J. E. Myers, J. P. Kass and G. O. Burr, *ibid.*, **18**, 107 (1941).

(12) R. W. Brauer and L. T. Steadman, *THIS JOURNAL*, **66**, 563 (1944).

The addition of alkali to the oxidized conjugated acids produced a marked increase in absorption in the longer wave lengths with the development of a maximum near 3750 Å. This maximum was not apparent in alkaline oxidized ethyl linolenate, linolenic acid, or elaidolinolenic acid. However, it was observed in the spectra of alkaline oxidized linoleates.⁴ The cause of this absorption or its significance is not known.

Summary

1. The changes in ultraviolet absorption spectra were followed during the oxidation of linolenic acid, ethyl linolenate, elaidolinolenic acid,

pseudoeleostearic acid, α -eleostearic acid, and β -licanic acid.

2. Oxidation of the non-conjugated trienes is accompanied by an increased absorption with the production of maxima at 2350 and 2750 Å. Oxidation of conjugated trienes is accompanied by decreased absorption in the region of 2600–2800 Å. and increased absorption in the region of 2300 and above 3200 Å.

3. Since the absorption spectra of purified fatty acids, conjugated or unconjugated, are not affected by cold alkali, it is concluded that the absorption bands which appear with autoxidation are due to oxygen-containing chromophores.

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Isolation and Structure of an Enzymatically Synthesized Crystalline Disaccharide, D-Glucosido-L-sorbose

BY W. Z. HASSID, M. DOUDOROFF, H. A. BARKER AND W. H. DORE

It has been shown that the bacterium *Pseudomonas saccharophila* contains a phosphorylase which catalyzes the reversible reaction: sucrose + inorganic phosphate \rightleftharpoons glucose-1-phosphate + fructose.^{1,2} Sucrose prepared from glucose-1-phosphate and fructose was isolated in crystalline form and shown to be identical with natural sucrose.³ In a preliminary report⁴ evidence was presented indicating that the bacterial enzyme preparation which synthesizes sucrose from glucose-1-phosphate and fructose, can also combine glucose-1-phosphate with L-sorbose or D-ketoxyllose to form the corresponding disaccharides. The present investigation is concerned with the preparation and the chemical constitution of the crystalline disaccharide formed from α -D-glucose-1-phosphate and L-sorbose under the influence of the partially purified phosphorylase from *Pseudomonas saccharophila*.

The procedure used for the isolation and crystallization of the disaccharide is a modification of that employed for the isolation and crystallization of synthetic sucrose. The empirical formula of the disaccharide obtained by elementary analysis is $C_{12}H_{22}O_{11}$. The compound does not reduce Fehling solution or alkaline ferricyanide. It has a sweet taste and gives a positive Seliwanoff reaction. It appears to be very slightly affected by invertase, but it is easily hydrolyzed with acid. The reducing value obtained after acid hydrolysis corresponds to a disaccharide consisting

of glucose and sorbose. A mixed glucosazone and sorbosazone was obtained from the products of hydrolysis. After fermenting out the glucose from the hydrolyzate, a pure sorbosazone could be prepared. The melting point of the carbohydrate is 178–180°. The specific rotation is $[\alpha]_D + 33^\circ$. Hydrolysis with acid changes the rotation to $+7.5^\circ$; this value agrees well with the expected rotation for an equimolar mixture of D-glucose and L-sorbose. The rate of acid hydrolysis is approximately twice that of sucrose.

The acetylated disaccharide has a rotation in chloroform, $[\alpha]_D + 38^\circ$. The molecular weight of this derivative determined by the Rast method is 578. This is 85% of the theoretical value for a completely acetylated disaccharide consisting of two hexose units. The low value is probably due to partial decomposition of the compound during the determination which involves heating to about 180°.

The fact that the disaccharide is non-reducing shows that the glucose and sorbose are linked through the carbonyl groups. Evidence that the L-sorbose exists in the disaccharide as sorbofuranose was obtained by oxidizing the compound with sodium periodate. In a disaccharide consisting of glucopyranose and sorbofuranose glycosidically united through positions 1 and 2 of the ketose and aldose monosaccharides, the glucose residue would possess three adjacent free hydroxyls, on carbon atoms 2, 3 and 4, and the sorbose residue would possess two free hydroxyls, on carbon atoms 2 and 3. On oxidation of such a disaccharide with periodate, the glucose residue should consume two moles of periodate and form one mole of formic acid, while the sorbose residue should consume one mole of periodate. A total

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(2) M. Doudoroff, *ibid.*, **151**, 351 (1943).

(3) W. Z. Hassid, M. Doudoroff and H. A. Barker, *This Journal*, **66**, 1416 (1944).

(4) M. Doudoroff, W. Z. Hassid and H. A. Barker, *Science*, **100**, 115 (1945).