heating, some material had appeared on the cooler parts of the flask. Further heating had no effect. The sublimate was then scraped from the walls of the flask and recrystallized from ethanol. Only a few milligrams of product melting at 236–237° was obtained for each gram of III used. Admixture with an authentic sample of *s*-diphenylurea produced no depression of the melting point.

Conversion of V into XII.—Two grams of V was dissolved in 125 cc. of dioxane and 10 cc. of water and 10 cc. of concentrated hydrochloric was then added. There was immediate formation of a yellow color upon addition of the acid. After standing for twenty-four hours, the solution was poured into 300 cc. of water.

After standing for forty-eight hours, the suspension resulting from this treatment had deposited yellow leaves. These were removed by filtration and washed with water. There was obtained in this manner 0.70 g. of dry yellow product, m. p. 166–176°. Two recrystallizations from methanol gave pink needles melting at 175–177°.

The pink color accompanying this product is as difficult to remove as that accompanying XI; its removal was accomplished by use of the same technique as that previously described for XI. This treatment gave very nearly white needles, m. p. 177–178°.

Anal. Calcd. for $C_{25}H_{23}NO_3$: C, 77.90; H, 6.01; N, 3.63. Found: C, 78.00; H, 6.74; N, 3.74.

Synthesis of XII.—Four-tenths gram of methyl α-acetyl-β-anilinohydrocinnamate and 0.50 g. of benzaldehyde were mixed and dissolved in 1 cc. of methanol with warming. One drop of concentrated sulfuric acid was added and the solution warmed for a few minutes longer, then allowed to cool. After two hours a small amount of precipitate had appeared. This was filtered off and recrystallized from dilute methanol; there was not enough material for two recrystallizations. There was obtained a white powder, m. p. 148–157°; admixture with XII gave a melting point of 152–164°.

Summary

1. It has been shown that the reaction of acetaldehyde with ethyl β-anilinocrotonate yields a dihydropyridine derivative.

2. It has been shown that the reaction of benzaldehyde with ethyl β-anilinocrotonate yields a dihydropyridine derivative and a tetrahydropyrimidine derivative and that the reaction of benzaldehyde with methyl β-anilinocrotonate yields a tetrahydropyrimidine derivative.

3. Several degradation products of these tetrahydropyrimidines have been isolated. The action of dilute hydrochloric acid upon these tetrahydropyrimidines yields compounds which are probably oxazines.

MINNEAPOLIS, MINNESOTA RECEIVED MAY 21, 1945

[CONTRIBUTION FROM THE DIVISION OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS]

Spectrophotometric Studies of the Oxidation of Fats. II. The Oxidation of Dienoic Fatty Acids

BY RALPH T. HOLMAN,[1] WALTER O. LUNDBERG[2] AND GEORGE O. BURR

The changes in fats which take place during autoxidation have been the subject of much study and many workers have proposed mechanisms for the oxidation of the component unsaturated fatty acids. Von Mikusch and Priest[3] have summarized briefly the theories and proposed mechanisms by which oxygen may attack the double bond. The mechanism whereby a four-membered ring peroxide is formed is favored with the more unsaturated fatty acids. The demonstration of oxido structures among the products of autoxidation of monoethylenic systems by Ellis[4] and Deatherage and Mattill[5] lends support to the mechanism leading to a three-membered ring peroxide containing tetravalent oxygen. Fahrion[6] first proposed a rearrangement of peroxides to form a ketol and Morrell[7] and co-workers have been able to identify ketols as products of oxidation of β-eleostearin.

Ellis[8] suggested that the addition of oxygen to the double bond yields an ene-diol. Scheiber[9] postulated the addition of oxygen to the unconjugated diene system at the active methylene group to form —CH=CH—CO—CH=CH—. Farmer[10,11] proposed a mechanism whereby oxygen attacks the carbon between the double bonds of the linoleic acid type system to yield a hydroperoxide. It will be noted that in the last three mechanisms the system retains its unsaturation after the addition of oxygen. In disagreement with these mechanisms is the work of Paschke and Wheeler[12] who found the decrease in iodine value roughly parallel to the increase in peroxide value during the oxidation of fatty acid esters.

Some work has been done relative to the spectroscopy of fat oxidations. Edisbury and co-workers[13] found an increase in the absorption of ling oil upon its exposure to air. In the oxidized sample there was an indication of an absorp-

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

(3) J. D. Von Mikusch and G. W. Priest, *Oil and Soap*, **18**, 50 (1941).

(4) G. W. Ellis, *Biochem. J.*, **30**, 757 (1936).

(5) F. E. Deatherage and H. A. Mattill, *Ind. Eng. Chem.*, **31**, 1425 (1931).

(6) W. Z. Fahrion, *Angew. Chem.*, **16**, 665, 697 (1903).

(7) R. S. Morrell and E. O. Phillips, *Fette u. Seifen*, **46**, 541 (1939).

(8) G. W. Ellis, *J. Soc. Chem. Ind.*, **45**, 193T (1926).

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(11) E. H. Farmer and D. A. Sutton, *J. Chem. Soc.*, 119 (1943).

(12) R. F. Paschke and D. H. Wheeler, *Oil and Soap*, **21**, 52 (1944).

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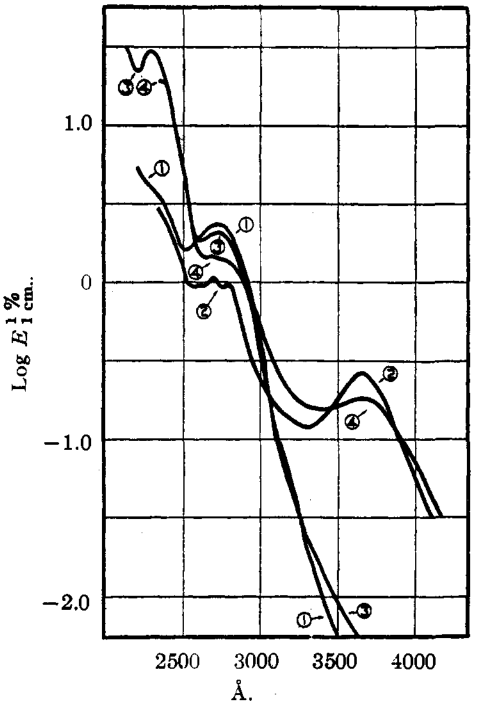

Fig. 1.—Ultraviolet absorption spectra of ethyl linolate: (1), ethyl linolate, P.V. = 5.6; (2), (1) in KOH; (3), (1) in air 63°, 70 hr., P.V. = 211.0; (4), (3) in KOH.

tion band near 2700 Å. Farmer's group[14] reported a general rise in the absorption spectrum of methyl docosahexaenoate with the disappearance of the band at 2700 Å. and an increased absorption at 2300 Å. when the substance was exposed to air. In a subsequent study Farmer and co-workers[15] repeated this work with the same result, and also reported that the absorption bands at 2300 and 2700 Å. in ethyl linolenate increased upon oxidation. A comparison of squalene and oxidized squalene showed a significant rise in the absorption upon oxidation, but no pronounced band appeared. From their studies these workers concluded that oxidation of unsaturated fatty acids is accompanied by conjugation of the isolated double bonds. Mitchell and Kraybill[16a] found that at the beginning of oxidation the absorption due to conjugation caused by bleaching, is reduced; however, as had been previously shown[16b] further oxidation of linseed oil is accompanied by increased absorption which does not have the fine structure characteristic of triene conjugation between carbon atoms. Brauer and Steadman[17] studied the course of the oxidation of β-eleostearic acid and found that as the oxidation proceeded the band at 2680 Å. disappeared. Since conjugated systems with accompanying light absorption may be produced in fats by saponification,[18,19] by distillation,[20] by debromination,[21] and by treatment with adsorbents,[16] as well as by oxidation, the appearance of weak bands in the spectra of fats need not be directly related to oxidative changes.

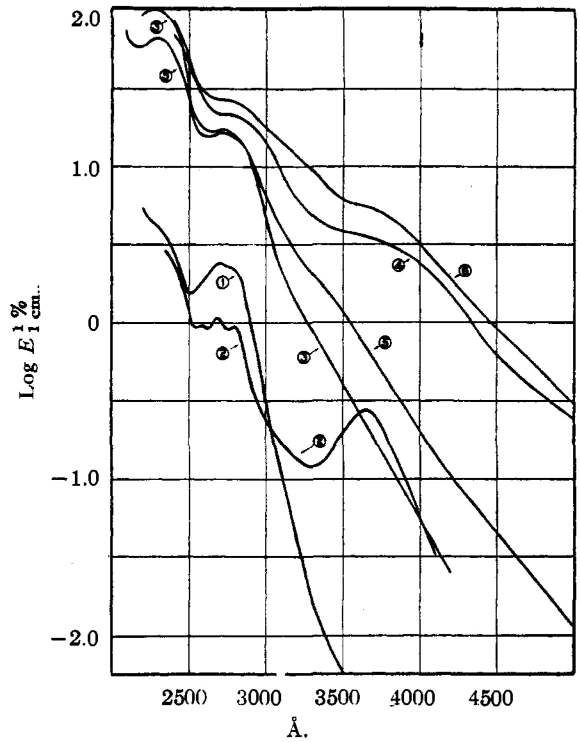

Fig. 2.—Ultraviolet spectra of ethyl linolate: (1), ethyl linolate, P.V. = 5.6; (2), (1) in KOH; (3), (1) in air 110°, 192 hr., P.V. = 38.3; (4), (3) in KOH; (5), (3) in vacuum 110°, 312 hr., P.V. = 0.5; (6), (5) in KOH.

It was shown in the first part of this study[22] that upon oxidation of oleic acid, ethyl oleate, and elaidic acid the absorption increased with a marked inflection at 2750 Å. and a maximum at 2300 Å. The following pages describe the changes in absorption spectra of the doubly unsaturated fatty acids.

Experimental

The ethyl linolate used in this study was prepared by the debromination procedure of Rollet.[23] The sample boiled at 180° (2 mm.) and showed a peroxide value of 5.6. The linoleic acid[24] used was prepared by repeated low temperature crystallization. Crotylideneacetone was prepared by the method of Meerwein[25] (n^{15}D 1.5136), and mesityl

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(14) E. H. Farmer and D. A. Sutton, *J. Chem. Soc.*, 122 (1943).

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(16) (a) J. H. Mitchell, Jr. and H. R. Kraybill, THIS JOURNAL, **64**, 988 (1942); (b) *Ind. Eng. Chem., Anal. Ed.*, **13**, 765 (1941).

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(24) Kindly supplied by Dr. J. B. Brown.

(25) H. Meerwein, *Ann.*, **358**, 85 (1909).

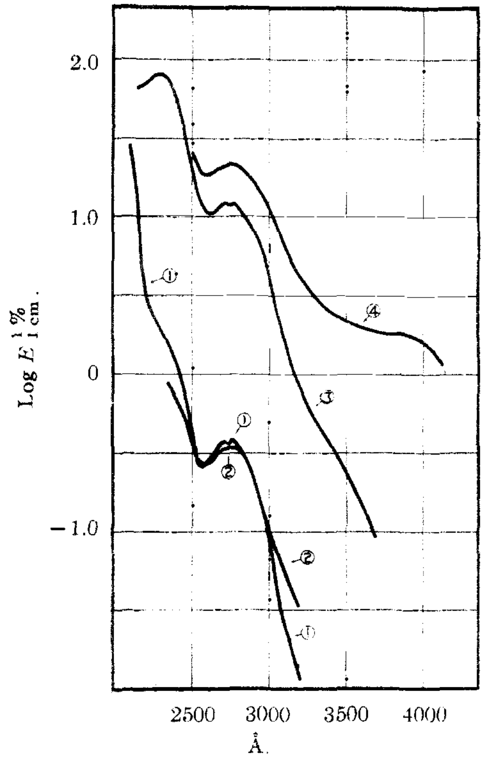

Fig. 3.—Ultraviolet absorption spectra of linoleic acid: (1), linoleic acid by low temperature crystallization; (2), (1) in KOH; (3), (1) in air at 63°, 96 hr., P.V. = 280.0; (4), (3) in KOH.

oxide and phorone were prepared by the method of Bayer.[26] β-Ionone was purchased from du Pont and acrolein and α-methylacrolein were purified from the Shell product. The 10,12-linoleic acid was prepared by Dr. J. P. Kass. The methods used in this study are the same as those in the study of the monoethenoic acids.[22]

Results and Conclusions

The sample of ethyl linolate used in this study unfortunately was not pure as shown by the peroxide value of 5.6. Its absorption spectrum showed a band in the region where conjugated trienes absorb strongly (Fig. 1). In alkali this band decreased slightly and fine structure appeared, but the absorption increased considerably at longer wave lengths with the appearance of a maximum at 3700 Å. Oxidation of the ester for seventy hours at 63° altered the spectral quality only slightly in either neutral or alkaline solution, except for a rise in absorption at 2350 Å. Treatment of the sample at 110° for 192 hours, conditions under which peroxides decompose readily and polymerization takes place, increased the absorption at 2700 Å. considerably (Fig. 2). Further heating in vacuum at 110° did not greatly change the absorption spectrum. None of the changes in absorption produced by oxidation are closely related to the peroxide value.

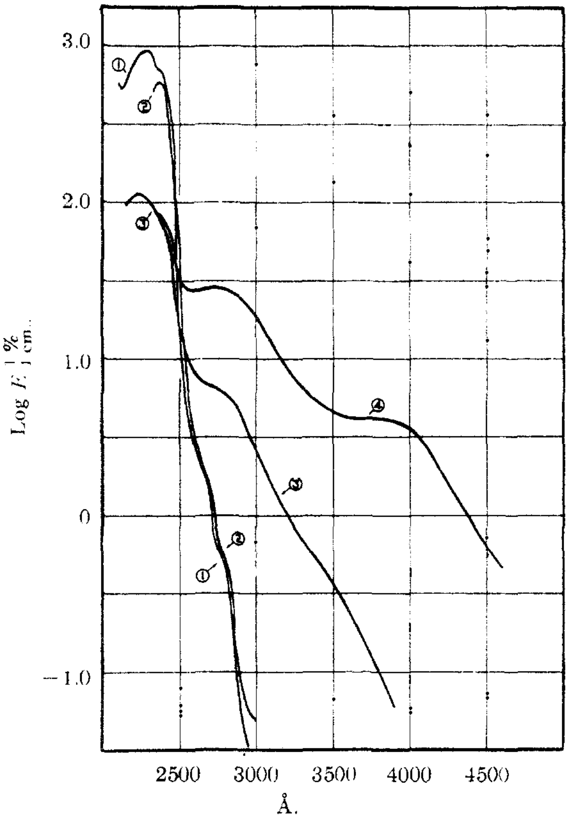

Fig. 4.—Ultraviolet absorption spectra of 10,12-linoleic acid: (1), 10,12-linoleic acid; (2), (1) in KOH; (3), (1) in air 63°, 45 hr., P.V. = 381.0; (4), (3) in KOH.

Linoleic acid, highly purified by twelve recrystallizations at low temperatures, showed almost identical spectra in alcohol and in alkali (Fig. 3). This indicates that cool alkaline solution has no effect upon the unconjugated doubly unsaturated system. Upon oxidation the acid showed a marked increase in absorption and the absorption in alkali at 2750 Å. was almost double that in alcohol, indicating that the band at 2750 Å. was due in part to enolizable substances (Table I).

Conjugated linoleic acid, whose spectrum is unchanged by alkali, becomes sensitive to alkali after oxidation (Fig. 4). The absorption at 2350 Å. due to the conjugated diene system decreased markedly with oxidation while the absorption above 2500 Å. increased. A band appeared at 2750 Å. which was accentuated in alkali. A pronounced band at 3800 Å. also appeared in the alkaline oxidized sample.

In summary, Figs. 1, 2, 3 and 4 show that like fresh oleic acid, the absorption spectrum of unoxidized linoleic acid (both natural and conjugated) is unaffected by alkali. However, the products of autoxidation behave wholly unlike those of oleic acid in that the absorption at 2700 Å. is only slightly affected by alkali while a pronounced band is produced at 3700 Å.

The type compounds used for the study of oleic acid and its oxidation products[22] included di-

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(26) A. Baeyer, *Ann.*, **140**, 297 (1866).

TABLE I

ABSORPTION MAXIMA FOR SAMPLES OF FRESH AND OXIDIZED LINOLATES

Substance		Treatment	P.V.	Alcohol $\lambda_{max.}$	Alcohol $E_{max.}$	KOH $\lambda_{max.}$	KOH $E_{max.}$
Ethyl linolate	1)	Fresh	5.6	2700 Å.	2.39	2575 Å.	0.97
						2675	1.08
						2800	0.95
						3650	0.27
	2)	(1) 63°, 70 hr.	211.0	2700	2.03	2700	1.48
						3600	0.19
	3)	(1) 110°, 192 hr.	38.3	2300	98.0	3700[a]	3.42
				2700	17.4		
	4)	(3) vacuum 110°, 13 days	0.5	2300	65.7	2800	26.0
				2700	16.7	3700[a]	5.34
Linoleic acid	5)	Fresh	...	2750	0.38	2750	0.34
	6)	(5) 63°, 96 hr.	279.0	2725	12.3	2750	21.8
				2775	12.2		
10,12-Linoleic acid	7)	Fresh	...	2300	916.0	2350	591.0
				2350[a]	725.0		
	8)	(7) 63°, 69 hr.	381.0	2300	107.0	2750	28.3
				2750[a]	6.30	3700[a]	4.10

[a] Denotes inflection.

hydroxy-, ketol-, diketo-, oxido-stearic acids, and their autoxidation products. In addition, acetone, acetonylacetone, acetylacetone and diacetyl were studied. None of these have spectral qualities in neutral and alkaline media similar to those of autoxidized linoleic acid.

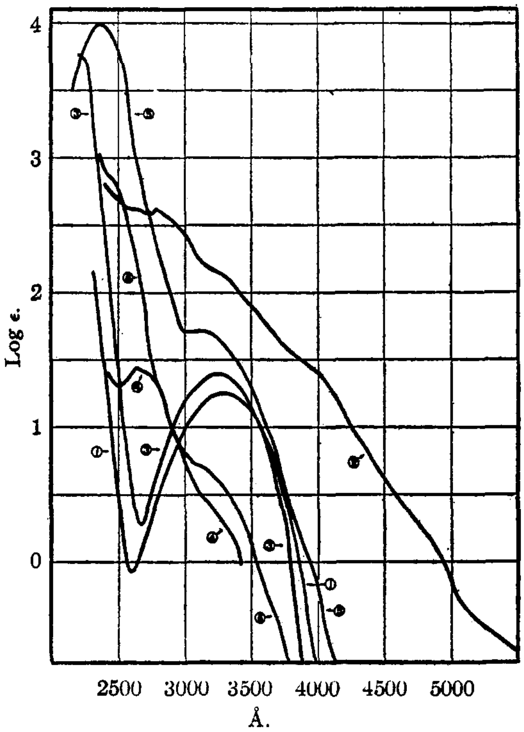


Fig. 5.—Spectra of reference compounds: (1), acrolein in alcohol; (2), (1) in KOH; (3), α-methylacrolein in alcohol; (4), (3) in KOH; (5), mesityl oxide in alcohol; (6), (5) in KOH.

Since it has been postulated that linoleic acid may add oxygen without destruction of the double bond (see above) other type compounds were studied in neutral and alkaline alcohol. These were:

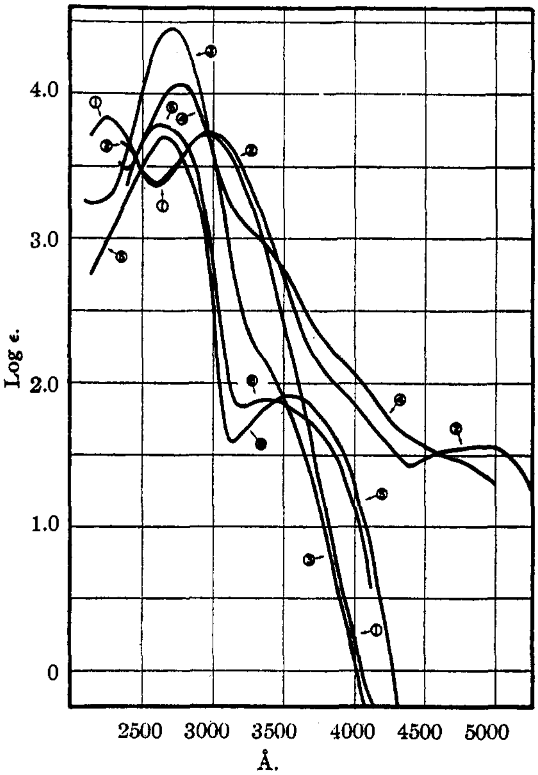
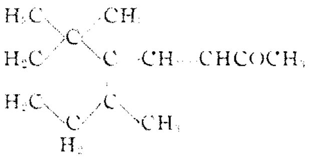


Fig. 6.—Spectra of reference compounds: (1), β-ionone; (2), (1) in KOH; (3), crotylideneacetone; (4), (3) in KOH; (5), phorone; (6), (5) in KOH.

Acrolein	$CH_2{=}CHCHO$
α-Methylacrolein	$CH_2{=}CH(CH_3)CHO$
Mesityl oxide	$(CH_3)_2C{=}CHCOCH_3$
Phorone	$(CH_3)_2C{=}CHCOCH{=}C(CH_3)_2$
Crotylideneacetone	$CH_3CH{=}CHCH{=}CHCOCH_3$
β-Ionone	

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 crotyildeneacetone. The former has the structure postulated by Scheiber[9] 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-methyl acrolein, 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.

MINNEAPOLIS 14, MINN. RECEIVED MARCH 14, 1945

[CONTRIBUTION FROM THE DIVISION OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF MINNESOTA]

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

BY RALPH T. HOLMAN,[1] WALTER O. LUNDBERG[2] AND GEORGE O. BURR

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.

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(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).

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

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