

went through a maximum of 0.056 at 30°, and decreased to approximately 0.01 in the range 145–180°. With wave length as the variable this ratio decreased from 0.079 at  $\lambda$ 2380 to 0.039 at  $\lambda$ 3340. With variations of intensity of radiation over a 2-fold range and pressure of aldehyde vapor over a 6-fold range, H<sub>2</sub>/CO remained substantially constant at 0.055.

3. The C<sub>2</sub>H<sub>6</sub>/CO ratio was found to remain

constant at 0.083 under all the conditions mentioned in (2) above.

4. A preliminary study revealed the presence of formaldehyde and glyoxal in the products of photolysis condensable at -80°.

5. The results indicate a photolysis mechanism for propionaldehyde similar to that postulated previously for acetaldehyde.

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[CONTRIBUTION FROM THE DIVISIONS OF PHYSIOLOGICAL CHEMISTRY AND OF ORGANIC CHEMISTRY, UNIVERSITY OF MINNESOTA]

## Spectrophotometric Studies of the Oxidation of Fats. I. Oleic Acid, Ethyl Oleate and Elaidic Acid<sup>1</sup>

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The course of chemical reactions taking place in the oxidation of fats by molecular oxygen has been the subject of much speculation and investigation. Many mechanisms have been proposed for the oxidation, and many substances have been detected in or isolated from oxidized fats which lend support to the various hypotheses, but little is known with certainty concerning the major intermediates and final products of the reactions. To supplement the existing knowledge of fat oxidations a spectrophotometric characterization of some of the possible products of fat oxidation has been attempted and the observations are presented here.

When carefully rendered lard was allowed to oxidize in air it was found that the ultraviolet absorption was increased greatly, and that the absorption in the region near 2700 Å. was roughly proportional to the peroxide value (Figs. 1, 2). However, when the peroxides were decomposed by blowing with superheated steam the absorption in this region showed an added increase. This indicated that the peroxides formed during the oxidation were not responsible for the absorption band appearing at 2700 Å., in agreement with Farmer's<sup>3</sup> observation that peroxides show only a low general absorption in this region. It should be noted that the 2700 Å. band appearing in most of the curves for oxidized fatty material is smooth, lacking the fine structure characteristic of conjugated trienes. However, this band appears as an inflection on the side of the much stronger band at 2300 Å., and much of the fine structure may be masked.

A study of the absorption spectra of the indi-

vidual unsaturated fatty acids was begun in an effort to ascertain which of these is involved in the change of absorption spectrum seen upon the oxidation of natural fats. In an attempt to determine what structures among the oxidation products were responsible for the observed increased absorption, studies were made of the spectra of compounds whose structures were identical with or related to possible intermediates in the oxidation of fatty acids. Primary attention was given to compounds that are probable or possible intermediates in the oxidation of the monoethenoic acids. Because some of the products of fatty acid oxidation are capable of enolization, the absorption spectra of all substances were also determined in strongly alkaline solution. In subsequent communications experiments with more highly unsaturated fatty acids will be summarized.

### Experimental

The instrument used in these studies was a Beckman quartz spectrophotometer. All determinations were made upon alcoholic solutions except in the cases of glycerides, for which peroxide-free ether was used as solvent. For determinations of spectra in alkaline solution the alcoholic solutions of the samples were added to equal volumes of 20% aqueous potassium hydroxide.

The oxidation of the fatty acids was accomplished by exposing them to air in an oven at 68° in the case of oleic acid and ethyl oleate, and at 63° in the case of elaidic acid. The sample vials were stoppered loosely with cotton to prevent the entrance of dust. In the case of lard, the sample was heated in a boiling water-bath and filtered air was bubbled through it at a constant rate. The decomposition of peroxides was accomplished by heating the samples at 110° for several days in vacuum-sealed ampules.

Peroxide determinations to indicate the progress of the oxidations were made according to King's<sup>4</sup> modification of Wheeler's method, and peroxide values are expressed as milliequivalents of peroxide per kilogram of sample.

The oleic acid<sup>5</sup> used in this investigation had a melting point of 12.5° and a peroxide value of zero; the ethyl oleate<sup>6</sup> a boiling point of 150° at 2 mm.,  $n_D^{20}$  1.4510, and a peroxide value of zero. The elaidic acid was prepared

(1) This paper represents part of 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) E. H. Farmer, H. P. Koch and D. A. Sutton, *J. Chem. Soc.*, 541 (1943).

(4) A. E. King, H. L. Roschen and W. H. Irwin, *Oil and Soap*, 10, 105 (1933).

(5) Prepared by Dr. F. L. Greenwood.

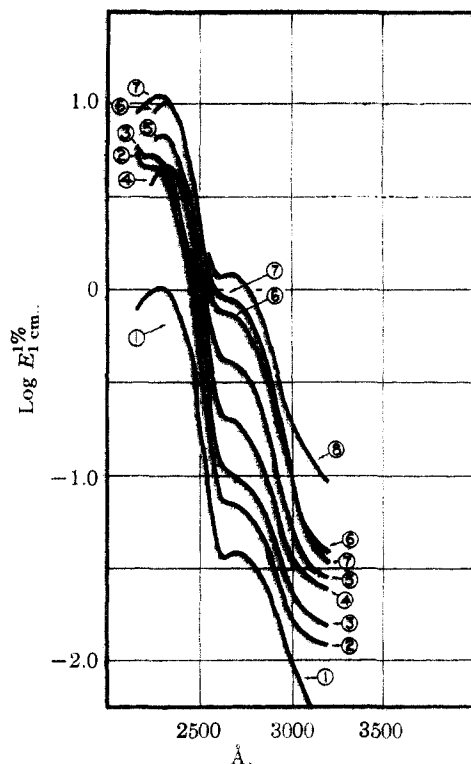


Fig. 1.—Absorption spectra of oxidized lard samples: (1) fresh lard, P.V. = 0.35; (2), (1) aerated at 100°, 5.3 hr., P.V. = 18.0; (3), (1) aerated at 100°, 6.5 hr., P.V. = 30.0; (4), (1) aerated at 100°, 7.0 hr., P.V. = 70.0; (5), (1) aerated at 100°, 12.8 hr., P.V. = 128.0; (6), (1) aerated at 100°, 16.3 hr., P.V. = 188.0; (7), (6) steam-blown at 100°, P.V. = 260; (8), (6) steam-blown at 200°, P.V. = 3.1.

according to Lyntenberg<sup>6</sup> and had a melting point of 42–43°. 9,10-Oxidostearic acid was prepared from the lower melting isomer of dihydroxystearic acid by the method of King,<sup>7</sup> and also from elaidic acid by the method of Atherton and Hilditch.<sup>8</sup> The former had a melting point of 54.2–54.7° and the latter had a melting point of 53–54°. The isomeric 9,10-dihydroxystearic acids<sup>9</sup> having melting points of 92–94° and 129–131° were prepared by the methods of Scanlan and Swern<sup>10</sup> and Le Seur,<sup>11</sup> respectively. 9-Keto-10-hydroxystearic acid<sup>9</sup> and 9-hydroxy-10-ketostearic acid<sup>9</sup> which had melting points of 72–73° and 73–75°, respectively, were prepared according to the method of King.<sup>12</sup> The 9,10-diketostearic acid<sup>9</sup> was prepared according to the method of Nicolet and Jurist<sup>13</sup> and had a melting point of 85–86°.

### Results and Discussion

As the oxidation of oleic acid proceeded it was found that the absorption in alcohol in the region of 2700 Å. did not change appreciably in the early stages (Fig. 3, solid lines). However, the ab-

(6) Lyntenberg, *Fett. chem. Um.*, **42**, 89 (1935).

(7) G. King, *J. Chem. Soc.*, 37 (1943).

(8) D. Atherton and T. P. Hilditch, *ibid.*, 204 (1943).

(9) Prepared by L. C. Behr.

(10) J. T. Scanlan and D. Swern, *THIS JOURNAL*, **62**, 2305 (1940).

(11) H. R. Le Seur, *J. Chem. Soc.*, **9**, 1313 (1901).

(12) G. King, *ibid.*, 1788 (1936).

(13) B. H. Nicolet and A. E. Jurist, *THIS JOURNAL*, **44**, 1136 (1922).

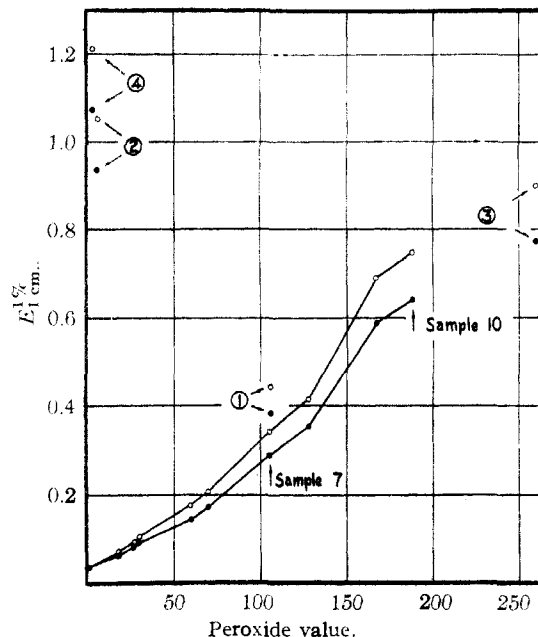


Fig. 2.—Peroxide value of oxidized lard vs. light absorption at 2650 and 2750 Å.: O—O, absorption of lard samples at 2650 Å.; ●—●, absorption of lard samples at 2750 Å.; (1), sample 7 blown with steam at 100°; (2), sample 7 blown with steam at 200°; (3), sample 10 blown with steam at 100°; (4), sample 10 blown with steam at 200°.

sorption in alkali increased progressively from the beginning of oxidation (Fig. 3, broken lines). After forty hours at 68° a sample was removed, sealed in vacuum, and heated eight days at 110°. The resulting product, reduced to a peroxide value of 1.4, absorbed much more strongly at 2700 Å. in alcohol and in alkali than its control with a peroxide value of 72. If it were assumed that the decomposition of peroxides gave rise to conjugated polyenes, the decomposition of 1.15% oleic acid peroxide (loss of peroxide in falling from P.V. 72.5 to 1.4) would give rise to about 1% conjugated diene or triene in maximum yields. The presence of 1% conjugated triene would give the mixture an  $E_{1\text{cm.}}^{1\%}$  of about 19 at 2700 Å. The observed extinction coefficients (2.0 and 1.0) at 2300 and 2700 Å. would be attained if 18 and 0.5% of the peroxides were converted to conjugated dienes and trienes, respectively. Again, if the increased absorption in alkaline solution found after peroxide decomposition were caused by formation of conjugated trienes, the trienes formed could account for only 10% of the peroxides decomposed. If all the increased absorption at 2700 Å. observed during the forty days autoxidation were due to formation of conjugated triene, the increased absorption would correspond to a 0.1% conjugated triene content. Likewise, if all the increased absorption in alkaline solution were ascribed to conjugated trienes, a maximum of 0.26% conjugated trienes could be present (for numerical values see Table I).

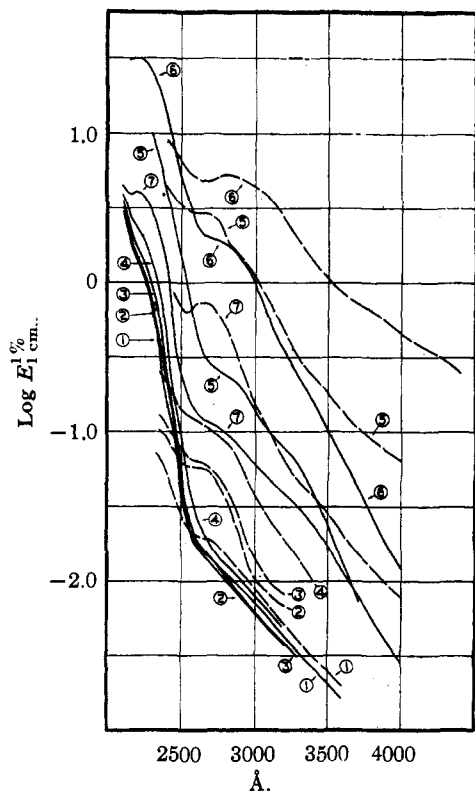


Fig. 3.—Ultraviolet absorption of oxidized oleic acid: (1), fresh oleic acid, P.V. = 0; (2), (1) in air 68° 16 hr., P.V. = 7.4; (3), (1) in air 68° 24 hr., P.V. = 19.4; (4), (1) in air 68° 40 hr., P.V. = 72.5; (5), (1) in air 63° 14 days, P.V. = 61.0; (6), (1) in air 63° 40 days, P.V. = 55.0; (7), (4) in vacuum 110° 8 days, P.V. = 1.4; ——— in alcoholic solution; ——— in KOH.

Upon oxidation of ethyl oleate the absorption in alcohol was found to increase only slightly although the sample reached a peroxide value of 725 (Fig. 4) and the absorption increased greatly in alkali. Heat treatment gave a picture similar to that of oleic acid.

The spectra of oxidized elaidic acid were measured periodically during the course of the autoxidation which was continued for fifty-one days (Fig. 5). The absorption rose as the oxidation proceeded, and in this respect the elaidic acid and oleic acid were similar, although the absorption by elaidic acid rose more rapidly during the early stages of the oxidation. In both cases it was observed that the greatest increases in absorption took place in the later stages of the oxidation accompanied by the decomposition of peroxides, indicating that the compounds which are responsible for the increased absorption are secondary products produced by the decomposition of the primary products. This is borne out by the increased absorption observed in those samples of fat or fatty acids which were subjected to higher temperatures in the absence of oxygen. It should be pointed out that the changes in absorption are,

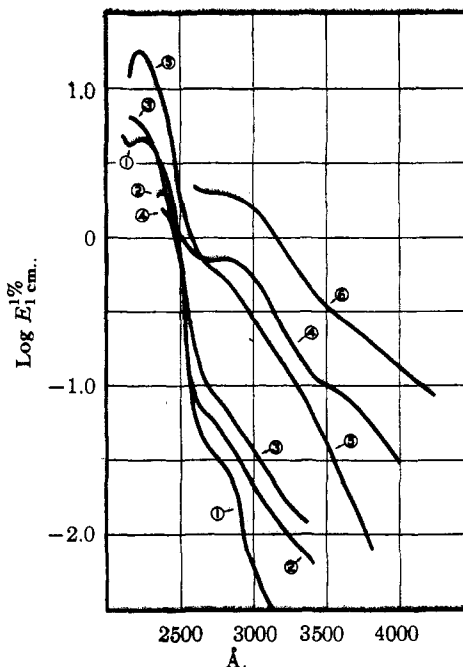


Fig. 4.—Ultraviolet absorption of ethyl oleate: (1), fresh ethyl oleate, P.V. = 0 (in alcoholic solution); (2), (1) in KOH; (3), (1) in air, 68°, 6 days, P.V. = 725 (in alcoholic solution); (4), (3) in KOH; (5), (3) in vacuum at 110°, 14 days, P.V. = 1.6 (in alcoholic solution); (6), (5) in KOH.

much greater in alkaline solution than in neutral alcohol and constitute a more sensitive test for oxidative changes especially in the early stages of the oxidation.

The absorption spectra of the dihydroxystearic acids show only an inflection at 2750 Å. but the general absorption is considerably greater than that of the oleates and elaidic acid (Fig. 6). The inflections may indicate the presence of small amounts of impurity which may also be responsible for the new band which appears in alkaline solution. The formation of dihydroxystearic acids in autoxidation of unsaturated acids could contribute to the increased absorption observed in the early stages but could not account for the more intense bands in strongly oxidized samples (Table I).

The isomeric ketols exhibit strikingly similar absorption spectra (Fig. 7). The absorption bands shown by these substances are of such intensity that the presence of a few per cent. of ketols in the oxidized fatty acids would be detectable. Upon enolization the ketols show a very strong band at 3100 Å. which was not observed in the spectra of oxidized oleic acid, ethyl oleate, or elaidic acid in alkali. It may then be concluded that 9,10-ketolstearic acids do not accumulate in more than traces in oxidized monoethenoic acids.

If diketostearic acid were present in oxidized fats to any considerable extent, its two absorp-

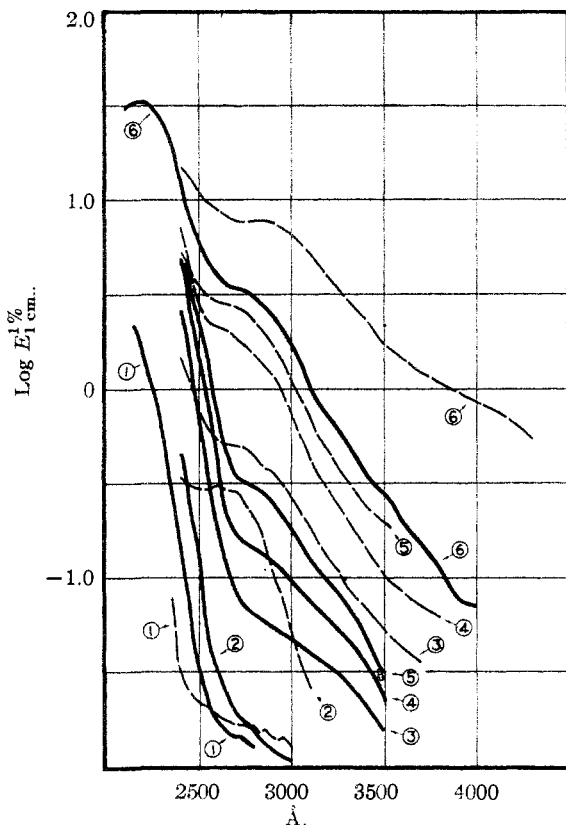


Fig. 5.—Ultraviolet absorption spectra of oxidized elaidic acid: (1), fresh elaidic acid; (2), (1) in air at 63°, 69 hr., P.V. = 50.0; (3), (1) in air at 63°, 144 hr., P.V. = 181.0; (4), (1) in air at 63°, 192 hr., P.V. = 238.0; (5), (1) in air at 63°, 260 hr., P.V. = 196.0; (6), (1) in air at 63°, 51 days, P.V. = 100.0; ——— in alcoholic solution; ——— in KOH.

tion maxima at 2750 and 4250 Å. should appear (Fig. 8). However, in very few of the oxidized acids which showed a maximum at 2750 Å. was the absorption strong enough in the visible region to be measured on the small samples available. In no case was there evidence of increasing absorption in the longer wave lengths as would be the case if detectable quantities of  $\alpha$ -diketo structures were present. Treatment of diketostearic acid with alkali increased the absorption greatly and two maxima appeared at 2600 and 3100 Å. No such dual bands were found in alkaline solutions of oxidized oleates or elaidic acid.

Among the oxidation products demonstrated in the oxidized ethylenic systems by Mattill and co-workers<sup>14</sup> and by Ellis<sup>15</sup> is the oxido derivative. The absorption spectra of the two samples of 9,10-oxidostearic acid prepared from elaidic and dihydroxystearic acids were found to be very similar. The absorption was low, and at no point did the absorptions of the two samples differ significantly. The accumulation of oxidostearic acid itself in

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

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

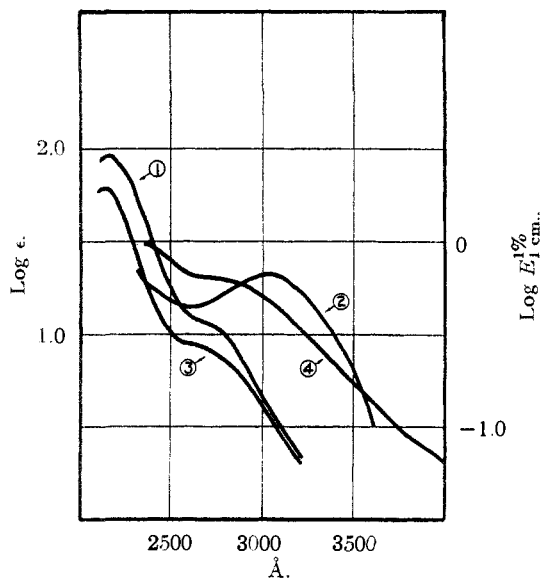


Fig. 6.—Ultraviolet absorption spectra of 9,10-dihydroxystearic acids: (1), low-melting isomer in alcohol; (2), (1) in KOH; (3), high-melting isomer in alcohol; (4), (3) in KOH.

oxidized fatty acids would serve only to increase the general absorption and could not be detected from the spectra (Fig. 9).

To learn whether the spectra of the derivatives

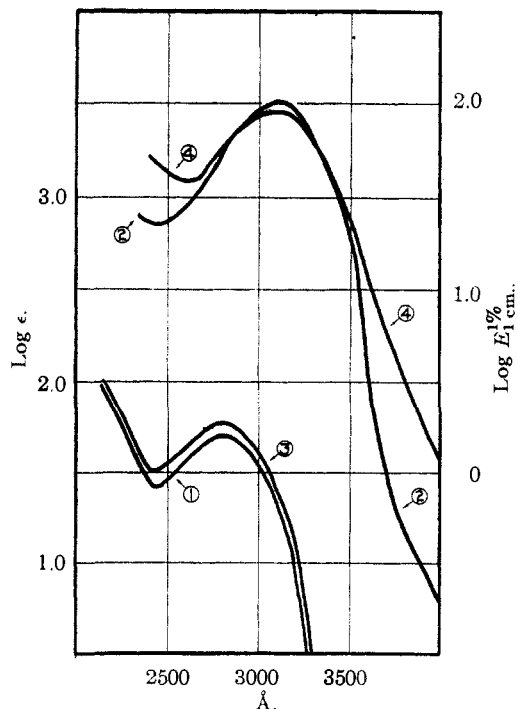


Fig. 7.—Ultraviolet absorption spectra of ketostearic acids: (1), 9-keto-10-hydroxystearic acid in alcohol; (2), (1) in KOH; (3), 9-hydroxy-10-ketostearic acid in alcohol; (4), (3) in KOH.

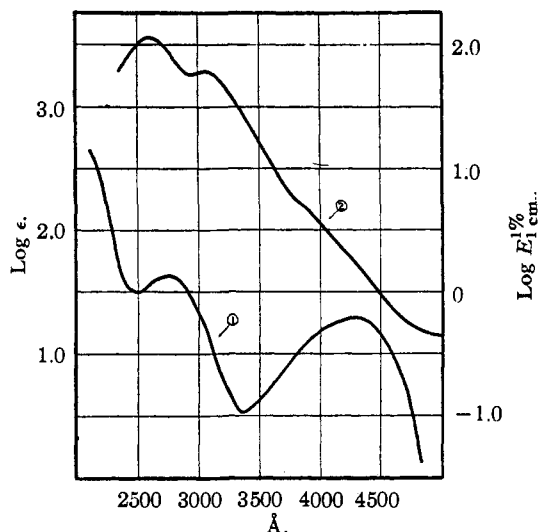


Fig. 8.—Ultraviolet absorption spectra of diketostearic acid: (1), in alcoholic solution; (2), in KOH.

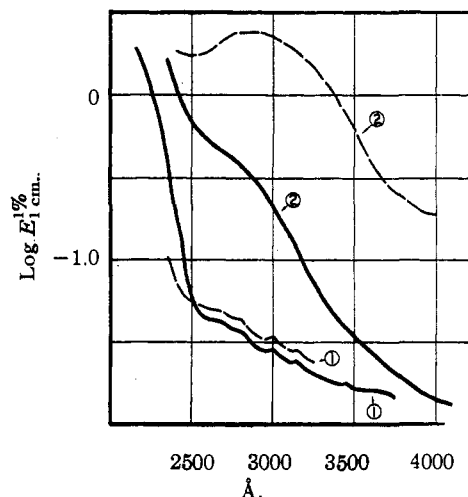
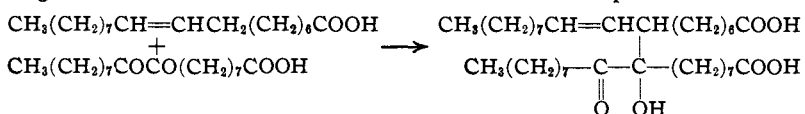


Fig. 9.—Ultraviolet absorption spectra of oxidostearic acid: (1), 9,10-oxidostearic acid (prepared from dihydroxystearic acid); (2), (1) in air at 81° for 9 days; ———— in alcoholic solution; - - - - in KOH.

of oleic acid are altered in the presence of oleic acid, these derivatives were dissolved in alcohol to which oleic acid was added in quantities of from ten to twenty times the quantity of derivative. The calculation of extinction coefficients was based upon the quantity of derivative present, corrections being made for the absorption due to the oleic acid present (Fig. 10). It is seen from the spectra of the mixtures of oleic acid with the diketostearic acid and ketolstearic acid that the character of these derivatives is not altered in the presence of oleic acid. However, in alkaline solution these mixtures exhibit strikingly similar spectra, which are similar to the ketolstearic acid alone in alkali. The mixture of diketostearic acid and oleic acid in alkali does not show the dual bands characteristic of diketostearic acid alone in alkali, but shows the single band characteristic of the ketol in alkali. The appearance of maxima at 3100 Å. in these mixtures where none appear in the spectra of alkaline oxidized fatty acids indicates that neither diketostearic acid nor ketolstearic acid accumulates in oxidized fats in more than traces.

The spectral similarity of the alkaline mixture of oleic acid and diketostearic acid to alkaline ketolstearic acid indicates the conversion of the diketo group to some structure which is spectrophotometrically equivalent to that of a ketol. A condensation of oleic acid and diketostearic acid might account for such a conversion:



The spectra of the mixtures of dihydroxystearic acid or oxidostearic acid with oleic acid showed no qualitative differences from those of the derivatives alone. This was also true for their alkaline

solutions. It may then be assumed that under these conditions no reaction takes place between the oleic acid and either the oxido- or dihydroxystearic acids which alter their spectra.

It was apparent that none of the compounds studied could account for the absorption maxima observed in the spectra of oxidized monoethenoic acids. The derivatives were next subjected to conditions suitable to promote autoxidation to learn whether these substances undergo changes under the conditions to which the fats were subjected in autoxidation. A somewhat higher temperature was chosen in order that all the derivatives (except dihydroxystearic acid) would be in a liquid state. After forty-six hours at 81° the ketolstearic acid and diketostearic acid had both changed, and their spectra had become closely alike (Fig. 10). This was borne out by the similarity of their spectra in alkaline solutions. The spectra of these two compounds in alkali after this treatment were also very similar to the spectra of alkaline solutions of their mixtures with oleic acid. Under the oxidative conditions the absorption at 4250 Å. increased for ketolstearic acid but decreased for diketostearic acid. This indicates that ketolstearic acid can be oxidized in air to form diketostearic acid, but that diketostearic acid under the same conditions is decomposed. The nature of the decomposition products of the diketostearic acid which have a spectrum like ketolstearic acid in alkali is not known. If diketo- or ketolstearic acids were formed in the oxidation of the monoethenoic acids and underwent further oxidative decomposition their products should then exhibit an absorption band in alkali near 3100 Å. Since no such band is observed, it may be concluded that these com-

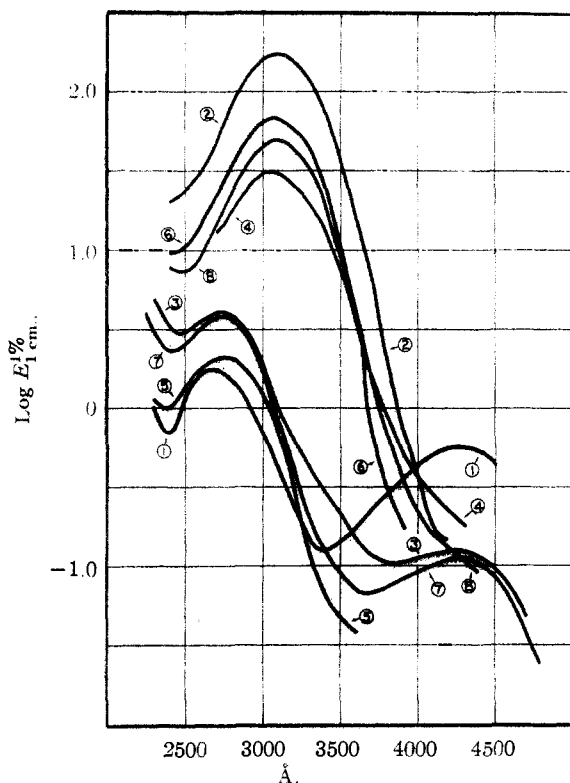
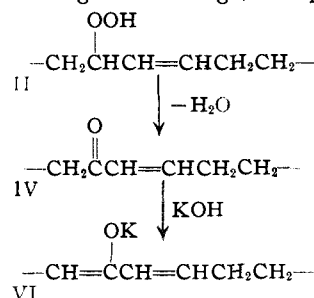


Fig. 10.—Ultraviolet absorption spectra of diketo- and ketol-stearic acids: (1), diketostearic acid in oleic acid; (2), (1) in KOH; (3), diketostearic acid in air at 81°, 46 hr.; (4), (3) in KOH; (5), ketolstearic acid in oleic acid; (6), (5) in KOH; (7), ketolstearic acid in air 81°, 46 hr.; (8), (7) in KOH.

pounds do not appear as oxidation products of monoethenoic acids in detectable quantities either as intermediates or final products.

Subjection of dihydroxystearic acid to 81° for 144 hours caused no significant changes in its spectra (Table I) indicating that it is not a likely precursor to the substance absorbing at 2750 Å. Oxidostearic acid under the same conditions for 216 hours did exhibit a rise in absorption and the alkaline solution of the oxidized oxidostearic acid showed a broad band from 2700 to 3100 Å. (Fig. 9). Under these conditions oxidostearic acid undergoes a change, the products of which may



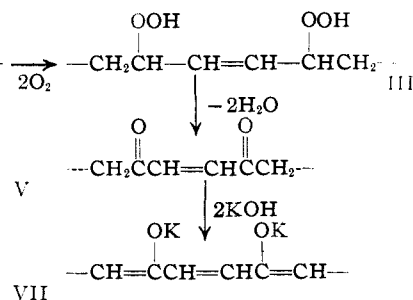
contribute significantly to the increased absorption observed in oxidized monoethenoic acids in

alkaline solution. Oxidized oxidostearic acid is the only substance tested which gives absorption spectra comparable to oxidized monoethenoic acids.

The presence of the absorption maximum at 2750 Å. in oxidized lard cannot be accounted for by the presence of any of the derivatives studied. This maximum lies in that portion of the spectrum in which the conjugated trienes absorb strongly. Mitchell and Kraybill<sup>16</sup> found that bleaching vegetable oils produced absorption which they attributed to triene conjugation. They proposed mechanisms by which unconjugated dienes could become conjugated through oxidation and dehydration. Farmer<sup>3</sup> postulated the formation of a hydroperoxide by the attack of oxygen at the carbon alpha to the double bond. Decomposition of such a compound could lead to formation of conjugated dienes and later trienes. It is known that such compounds as crotylidene-acetone, quinones and  $\beta$ -ionone, in which the carbonyl group is a part of a triply conjugated system, show strong absorption maxima near 2750 Å. It should be pointed out that triply conjugated systems containing oxygen do not show the fine structure exhibited by the spectra of conjugated trienes. It is also unlikely that conjugated trienes could be wholly responsible for the maxima appearing in oxidized fats at 2750 Å. because cold alkali treatment such as is used on the oxidized fatty acids causes no change in the absorption spectra of pure unsaturated fatty acids, conjugated or unconjugated.

It is unfortunate that the study could not be extended to include the region where the conjugated dienes absorb strongly, but reliable measurements in alkali could not be made below 2400 Å. because the hydroxyl group begins to absorb strongly in this region. The measurements made on the alcoholic solutions indicate that during the oxidation the absorption at 2350 Å. increased appreciably, presumably due to the formation of conjugated dienes.

If oxygen attacks the carbon alpha to the double bond of an ethylenic system during autoxidation, decomposition of the peroxides may lead to compounds containing oxygen which absorb ultraviolet light in the regions where conjugated dienes and trienes absorb. Possible courses of these reactions are



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

TABLE I  
 ABSORPTION AT 2750 Å.

Substance	Treatment	P.V.	$E_{1\text{cm}}^{1\%}$ at 2750 Å.		Enolization ratio $\frac{\text{Alkali E}}{\text{Neutral E}}$
			In alcohol	In KOH	
Lard in ether	(1) Fresh	0.35	0.036		
	(2) Aerated 100° 3.5 hr.	18	.063		
	(3) Aerated 100° 4.5 hr.	20	.091		
	(4) Aerated 100° 9.0 hr.	70	.172		
	(5) Aerated 100° 12.8 hr.	128	.354		
	(6) Aerated 100° 18.0 hr.	188	.640		
	(7) (6) blown 100° with steam	260	.775		
	(8) (6) blown 200° with steam	3.1	1.07		
Oleic acid	(1) Fresh	0	0.013	0.017	1.31
	(2) In air 68° 16 hr.	7.4	.012	.044	3.66
	(3) In air 68° 24 hr.	19.4	.013	.050	3.85
	(4) In air 68° 40 hr.	72.5	.016	.102	6.37
	(5) In air 63° 14 days	61	.251	2.64	10.50
	(6) In air 63° 40 days	55	1.89	5.12	2.71
	(7) (4) in vacuum 110° 8 days	1.4	0.103	0.610	5.92
Ethyl oleate	(1) Fresh	0	.037	.056	1.51
	(2) In air 68° 144 hours	725	.083	.720	8.68
	(3) (2) in vacuum 110° 14 days	1.6	.595	1.975	3.32
Elaidic acid	(1) Fresh	....	.014	0.016	1.14
	(2) In air 63° 24 hours	18	.016	....	....
	(3) In air 63° 69 hours	50	.025	.251	10.00
	(4) In air 63° 144 hours	181	.069	.489	7.10
	(5) In air 63° 192 hours	238	.152	2.49	16.4
	(6) In air 63° 11 days	196	.320	2.54	7.94
	(7) In air 63° 51 days	100	3.23	7.66	2.37
9,10-Dihydroxystearic acid (1) Fresh (m. p. 92-94°)	....	....	0.342	0.634	1.83
	(2) (1) In air 81° 144 hours	....	.389	.665	1.71
9,10-Dihydroxystearic acid (m. p. 129-131°); fresh	....	....	.246	.497	2.02
9-Hydroxy-10-ketostearic acid; fresh	....	....	1.82	49.0	27.03
9-Keto-10-hydroxystearic acid (1) Fresh	....	....	1.51	47.1	31.10
	(2) In air 81° 46 hours	....	3.84	18.4	4.79
Diketostearic acid (1) Fresh	....	....	1.37	80.0	58.30
	(2) In air 81° 46 hours	....	3.94	14.3	3.63
Oxidostearic acid (1) From elaidic acid	....	....	0.040	0.041	1.02
	(2) From dihydroxystearic acid	....	.038	.044	1.16
	(3) (2) In air 81° 216 hours	....	.431	2.28	5.30
Acetone	....	....	2.35	1.725	0.74
Acetylacetone	....	....	2.83	138.0	48.80
Acetylacetone	....	....	1148.0	35.8	0.032
Diacetyl	....	....	2.13	202.0	94.9

The presence of the 2750 Å. maximum observed in oxidized fatty acids may be explained by the formation of compounds such as V, or its enolic form which would also contain a conjugated triply unsaturated system. The presence of compound V in an oxidized fat would account for a band at 2750 Å. which is altered by alkali (VII).

In the last column of Table I are given the ratios of absorption in alkali to absorption in alcohol at 2750 Å. If a single absorptive compound is being formed during fat oxidation the enolization ratio should be a constant. An examination of the data leads to the following conclusions:

(1) The absorptive material in the fresh preparation of monoethenoic acids is relatively insensitive to alkali and there is only a slight increase in absorption in an enolizing medium.

(2) In the early stages of autoxidation the enolization ratio increases to values as high as 16.

(3) In the later stages of autoxidation when peroxide values are decreasing (destruction of peroxides is more rapid than formation), the enolization ratio decreases while total absorption continues to increase. This indicates that at least two types of chromophores are being produced, a condition which greatly complicates the spectroscopic evaluation of rancidity.

(4) The absorption of dihydroxystearic acid at 2700 Å., either fresh or exposed to air, is only slightly increased by alkali and could not account for the high enolization ratios in slightly oxidized monoethenoic acids.

(5) Both the ketol- and diketostearic acids have high enolization ratios which decrease with autoxi-

dation. Hence, by this measure their presence could account for the spectral behavior of the autoxidizing oleic acid. For reasons cited earlier neither satisfies all other conditions found in the oxidized fatty acids.

(6) Oxidostearic acid increases its enolization ratio upon autoxidation and in this regard is not at all like the autoxidized fatty acids

(7) Of the last four reference compounds in Table I acetylacetone and diacetyl have low absorption in alcohol and high enolization ratios reacting very much like the ketol- and diketostearic acids. Acetylacetone, on the other hand, reacts wholly unlike oxidized fats or the above fatty acid derivatives.

### Summary

1. The ultraviolet absorption spectra of lard in various stages of autoxidation are presented, showing that as oxidation proceeds the absorption increases, and in the region of 2750 Å. this increase is roughly proportional to the peroxide content. The increase in absorption is not due to peroxide.

2. The absorption spectra of partially oxidized oleic acid, ethyl oleate, and elaidic acid have been studied. The mild oxidation of these compounds

increases their absorption only slightly, but prolonged oxidation causes marked increases in the absorption.

3. Dihydroxystearic acids and oxidostearic acid cannot account for the absorption bands observed in oxidized fatty acids.

4. Although the absorption of the diketostearic acid and ketolstearic acids show absorption bands in the region of 2750 Å. they do not account for the absorption of oxidized fats in this region.

5. Ketols and  $\alpha$ -dicarbonyls do not accumulate in oxidized monoethenoic acids in more than trace amounts.

6. Alkaline treatment of autoxidized ketol- or diketostearic acids or alkaline treatment of mixtures of these compounds with oleic acid leads to products which are spectroscopically very similar.

7. It is suggested that the increased absorption found in oxidized fats is due in part to conjugated unsaturated systems containing carbonyl groups, or to conjugated polyenes formed by enolization of these systems.

8. During the oxidation monoethenoic acids show increased absorption at 2350 Å. indicating the probable formation of conjugated dienes.

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## Acceleration by Electrolytes of Alkaline De-esterification of Pectin

BY HANS LINEWEAVER

The observation that electrolytes accelerate the alkaline de-esterification of pectin was reported in an abstract on the effect of electrolytes on the enzymic de-esterification of pectin.<sup>1</sup> This note gives the results of the non-enzymic, alkaline de-esterification studies.

Investigations of reactions between ions have shown that increasing the ionic strength causes an increase in the rate of reaction between ions of like sign.<sup>2</sup> In the hydrolysis of simple esters, where a reaction between a neutral molecule and an OH<sup>-</sup> ion is involved, an increase in the salt concentration has only a very small accelerating effect (about 10% in 0.1 M solution<sup>3</sup>). In the case of pectin, however, a considerable acceleration due to a primary salt effect might be expected, since the reaction is between ions of like sign (that is, provided the negative charges in pectin are close enough to the ester bonds to interact with the OH<sup>-</sup> ions as they approach the pectin molecule). The formation of soluble ion complexes,

such as are formed between polyvalent cations and organic acids, also might result in an increased rate of hydrolysis greater than that due merely to change in ionic strength (*e. g.*, the increase in the rate of reaction of bromacetate and thiosulfate in the presence of polyvalent cations in aqueous media has been explained by the formation of complex ions<sup>4</sup>).

The results reported in Table I verify the expectation and show that electrolytes increase the rate of de-esterification of pectin as much as 400% at pH 9. Similar results were obtained at other pH's above 6. Also, McCready, *et al.*,<sup>5</sup> of this Laboratory reported that electrolytes accelerate the rate of de-esterification of pectin in ammonium hydroxide solution at pH 10.9.

Table I shows further that at equal ionic strengths calcium chloride causes a greater acceleration of hydrolysis than sodium chloride. This greater effectiveness of calcium may be attributed, of course, to ion complex formation between the divalent calcium cations and the pectin anions or to the inadequacy of the principle

(1) H. Lineweaver and G. A. Ballou, *Proc. Fed. Am. Soc. Exptl. Biol.*, **2**, 66 (1943); *Arch. Biochem.*, **6**, 373 (1945).

(2) S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p. 423.

(3) E. A. Moelwyn-Hughes, "The Kinetics of Reactions in Solution." Oxford Press, 1933, p. 184.

(4) Ref. 2, p. 429; V. K. LaMer and M. E. Kammer, *THIS JOURNAL*, **57**, 2662 (1935).

(5) R. M. McCready, H. S. Owens and W. D. Maclay, *Food Industries*, **16**, 794 (1944).