ture was cooled. The liquor was decanted and the residual iron mud was extracted with acetone. The acetone was distilled off *in vacuo*. The residue was dissolved in hot absolute ethanol and charcoaled. On cooling the alcoholic solution, fine white needles of the 4-aminophenyl-2'-aminothiazolyl-5' sulfone formed and were filtered off; m. p. 219–221°, with decomposition; yield, 35 g. (85%).

Summary

The preparation and some of the chemical characteristics of 4-aminophenyl-2'-aminothiazolyl-5' sulfone (Promizole) and its derivatives have been described.

Evidence is presented in support of the conclusion that under the influence of heat and acetic anhydride the 4-nitrophenyl sulfenyl group migrates from the amine of 2-aminothiazole to the 5-position on the thiazole ring.

DETROIT, MICH.

RECEIVED JANUARY 8, 1945

[CONTRIBUTION FROM THE DEPARTMENTS OF PHYSIOLOGICAL CHEMISTRY AND PHYSICS OF THE UNIVERSITY OF MINNESOTA]

Extreme Ultraviolet Absorption Spectra of the Fatty Acids[1,2]

BY I. I. RUSOFF, J. R. PLATT,[3] H. B. KLEVENS[4] AND G. O. BURR

The fatty acids offer one of the best series of compounds available for study of the effects of double bonds upon light absorption. Well-known members of the series range from zero to four unconjugated double bonds. There are also several distinct conjugated systems and by elaidinization the *trans*-configuration can be compared with the *cis*.

In several recent reviews[5,6,7] absorption data for numerous simple aliphatic compounds have been assembled and the effects of unsaturation, conjugation and geometric configuration discussed. However, only recently a fairly complete study of the far ultraviolet absorption by purified fatty acids has been made.[8] The work of Barnes *et al.*, extends the curves of the saturated acids, oleic, linoleic, linolenic and arachidonic, to 2100 Å. where the effect of additional double bonds is so marked that extinction coefficients of oils may be used in the calculation of composition.

However, at the limit of the above work (2100 Å.) none of the curves of the unsaturated fatty acids has reached a peak. A compilation of the spectral data for the unconjugated fatty acids and oils shows that in most of the work done to date the curves stop at or above 2200 Å.[9] It was thought desirable to extend these measurements into the Schumann region in order to get a complete picture of light absorption by these biologically important natural products. In the present paper the absorption curves of the unconjugated fatty acids have been extended to 1700 Å.; in addition a number of conjugated systems and geometric isomers have been included.

Experimental

Description of Instruments.—The fluorite vacuum spectrograph system employed in the studies presented in this paper was similar to that described by Scheibe.[10] A diagram of the essential parts of the apparatus is shown in Fig. 1. The light source (L) is a modified Urey type hydrogen discharge tube operating at approximately 0.8 ampere from the secondary of a 4000 v., 5 K. V. A. transformer. Light from the discharge tube (L) passes through the absorption cell (C) which is formed by the gap between two lithium fluoride windows which are permanently sealed to the hydrogen tube and the spectrograph, respectively. The window on the spectrograph is sealed with wax to a collar which, in turn, is sealed to an end-plate and attached to the spectrograph by a flexible bellows which permits easy adjustment and alignment of the cell. Three springs are connected to the end-plate of the spectrograph and the water jacket of the Urey tube. By adjusting the tension of the springs, the two windows are pressed uniformly against a copper spacer which separates them by a distance of 0.026 cm. During a test exposure the solution was continuously admitted into the cell through a capillary

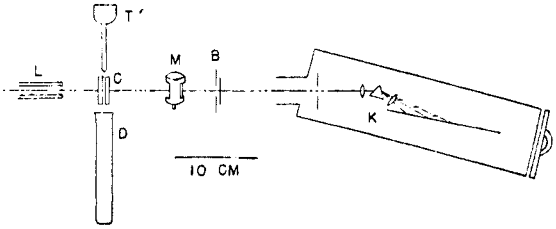

Fig. 1.—Vacuum spectrograph and accessories used in photographing the absorption spectra.

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(1) The experimental data are taken from a thesis submitted to the faculty of the Graduate School of the University of Minnesota by I. I. Rusoff in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) This work was supported by grants from the Rockefeller Foundation, the National Live Stock and Meat Board, and the Graduate School of the University of Minnesota. The authors are indebted to Professors Joseph Valasek and Elmer S. Miller (deceased) for their advice and criticisms; to J. B. Brown for a sample of methyl arachidonate and recrystallized linolenic acid; to J. Nichols for the preparation of debrominated linolenic and linoleic acid; to J. P. Kass for samples of elaidic acid, elaidolinolenic acid, the conjugated isomers of linoleic acid, and pseudoeleostearic acid; to W. M. Lauer for 2-heptadecenoic acid. They also express their thanks to F. Thurston for assistance with the laboratory work.

(3) Present address: Northwestern University, Evanston, Illinois.

(4) Present address: University of Chicago, Chicago, Illinois.

(5) E. P. Carr and H. Stücklen, "Proc. of the Seventh Summer Conference on Spectroscopy and its Applications," John Wiley and Sons, Inc., New York, N. Y., 1940, p. 128.

(6) K. Dimroth, *Angew. Chem.*, **52**, 545 (1939).

(7) G. O. Burr and E. S. Miller, *Chem. Rev.*, **29**, 419 (1941).

(8) R. H. Barnes, I. I. Rusoff, E. S. Miller and G. O. Burr, *Ind. Eng. Chem., Anal. Ed.*, **16**, 385 (1944).

(9) I. I. Rusoff, "Spectral Absorption Characteristics of Lipids," Thesis, University of Minnesota, 1943.

(10) G. Scheibe, *Z. physik. Chem.*, **B5**, 355 (1929).

thistle tube (T). This procedure minimized the photodecomposition of the solute and evaporation of the solvents. With the thickness of cell used, surface tension held the solution in place. A test-tube (D) caught the drainings. After making an exposure the remaining liquid was blown out with an air blast and the cell cleaned by rinsing a few times with the solvent. Detailed drawings of this attachment are given in an earlier paper.[14]

After leaving the cell, the light passes through a photometric rotor (M) described by Platt and co-workers[11] and a small camera shutter (B) which is controlled from outside the vacuum can by means of a small flexible arm. The slit and the optical system (K) of the fluorite spectrograph have been described previously by Cario and Schmitt-Ott.[12]

This instrument takes four exposures on a plate 0.75 by 4 inches, which is racked downward between exposures by an electro-magnet controlled from outside. The plate is put in and taken out of the spectrograph in total darkness through a hinged door sealed with vacuum putty. A vacuum can be obtained in two minutes with the ordinary vacuum pump because of the small volume of the spectrograph. Eastman 103-0 and I-O ultraviolet sensitized spectrographic plates were used throughout this work. These were developed and fixed in temperature controlled trays for a definite time according to standard procedures. The densities were measured on a densitometer of the Kipp and Zonen type which contained a thermopile-galvanometer detector.

The absorption curves presented here are plotted in terms of the logarithm of the extinction coefficient ϵ, defined as $\epsilon = E/cd$, where E = optical density = log incident light/transmitted light; d = length of the cell in cm. and c = concentration of solution in moles per liter. For oils, and other substances of unknown molecular weights, the concentration has been expressed as grams per 100 cc. of solution, in which case the coefficient is designated as $E^{1\%}_{1\text{cm.}}$

Method.—To determine extinction coefficients four equal exposures were taken as quickly as possible, the first two through the solvent with the rotor set at the 22° 35′ opening and the 52° 48′ opening, respectively. The solution was then interposed and the third and fourth exposures were taken with the rotor opening set at 78° 55′.

The amount of light passing through the rotor is proportional to the size of the opening. If the photographs through the solutions (3 and 4) have the same optical density as exposure 1, $E = \log (78° 55'/22° 34') = 0.544$. But if exposures 3 and 4 have the same density as exposure 2, $E = \log 78° 55'/52° 48' = 0.174$. The density values are read in the straight line portion of the H–D curve and the extinction coefficients are obtained graphically by interpolation. Optical densities ($\log I_0/I_x$) from 0.20 to 1.20 were preferred but in some cases it was necessary to use values up to 2.00.

In this study the fatty acids were weighed into 10-ml. volumetric flasks and were immediately diluted with solvent. The solution was allowed to come to room temperature and made up to the mark with solvent. Dilutions were made as follows: $C/2$, $C/4$, $C/10$, $C/20$, $C/40$, etc. Each solution was then analyzed spectrographically until a concentration was reached at which the maximum absorption could be measured. These dilutions were of such an order that several determinations of the extinction coefficient could be obtained for each wave length from the different plates.

The extinction coefficients at wave lengths above 2100 Å. were checked on a medium Hilger (E 31) quartz spectrograph. A 0.5 cm. absorption cell was used with a modified Urey type hydrogen discharge tube as a light source. Plates were taken on the spectrograph and calibrated by means of a step slit similar to that described by Duffendack and Wolfe.[13] A rotating sector disk was used to reduce the intensity of solvent exposures. The values, in close agreement with those obtained with the fluorite spectrograph, are designated in the following graphs by distinctive markings. Specially purified *n*-heptane, ethyl alcohol and iso-octane were used as solvents.[14]

Results

Effect of Solvents on Saturated Fatty Acids.—Recrystallized myristic acid was dissolved in ethyl alcohol, iso-octane and *n*-heptane. Its absorption in the three solvents is shown in Fig. 2. As there were no differences between the octane and heptane solutions, the extinction coefficients for the acid in these two solvents were averaged together. The polar solvent, alcohol, tends to shift the absorption maximum to the longer wave lengths and to lower the absolute values of the extinction coefficients. This observation agrees with that of Ley and Arends.[15]

Effect of Increasing Molecular Weight on the Absorption of Saturated Fatty Acids.—Redistilled glacial acetic acid, caprylic acid (Eastman Kodak Company) and recrystallized myristic acid were dissolved in *n*-heptane. Absorption curves for these three acids are presented in Fig. 3.

The acetic acid curve is lower than that of caprylic or myristic acid. It is also somewhat lower than the curves given by Henri[16] and Ley and Arends.[15] However, it is clear that in heptane all of the saturated fatty acids have one broad band with the maximum at about 2050 Å. and another region of very intense absorption beginning at 1850 Å. and still rising steeply at 1730 Å., the limit of these measurements.

The Effect of Increasing Unsaturation on the Absorption of Unconjugated Fatty Acids.—Oleic acid was made from olive oil by low temperature crystallization according to the procedure of Brown.[17] It had an iodine number (Wijs) of 88 (theory 90) and a melting point of 13.0° (recorded 13.16). It was estimated from the iodine number to be 97.5% pure. Probably the only impurity of consequence was a small amount of saturated fatty acids. Its maximum absorption occurs at about 1830 Å. (Fig. 4).

Linoleic acid, iodine number 181 (theory 181), was made by debromination of tetrabromostearic acid obtained from corn oil. Its absorption maximum is at about 1900 Å. (Fig. 4). The weak band at 2300 Å. may be attributed to the presence of some conjugated material produced during preparation of the acid. Since the conjugated acids have a log ϵ (2300 Å.) of about 4.5 (Fig. 10) there could not be more than a half of one per cent. present. This probably is a high estimate.

A sample of linolenic acid which had been prepared by the low temperature crystallization method was next studied. The acid had been

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(11) J. R. Platt, H. Clark, A. A. Cohen and P. A. Caldwell, *Rev. Sci. Inst.*, **14**, 85 (1943).

(12) G. Cario and H. D. Schmitt-Ott, *Z. Physik*, **69**, 719 (1931).

(13) O. S. Duffendack and F. H. Wolfe, U. S. Patent 1,979,964.

(14) J. R. Platt, I. I. Rusoff and H. B. Klevens, *J. Chem. Phys.*, **11**, 535 (1943).

(15) H. Ley and B. Arends, *Z. physik. Chem.*, **17**, 177 (1932).

(16) V. Henri, "Études de Photochemie," Gautier Villars et Cie., Paris, 1919.

(17) J. B. Brown, *Chem. Rev.*, **29**, 333 (1941).

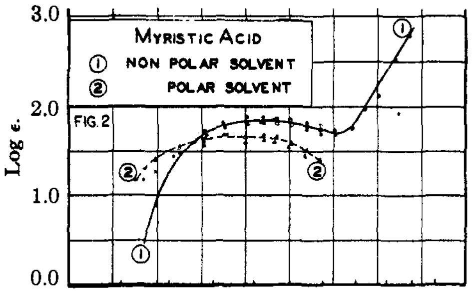

Fig. 2.—Absorption spectra of myristic acid in polar and non-polar solvents. Curve 1 is made from the average of values obtained with iso-octane and *n*-heptane; curve 2 in 95% ethyl alcohol.

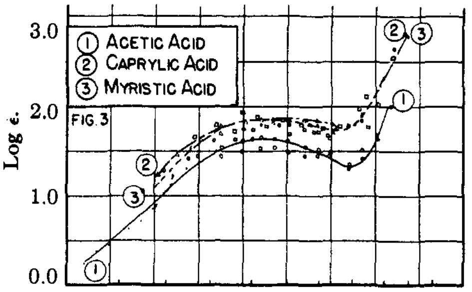

Fig. 3.—Absorption spectra of acetic, caprylic and myristic acids.

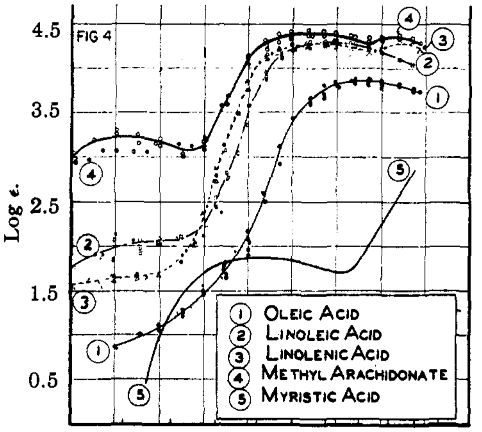

Fig. 4.—Absorption spectra of several unsaturated fatty acids compared with myristic acid (all in heptane).

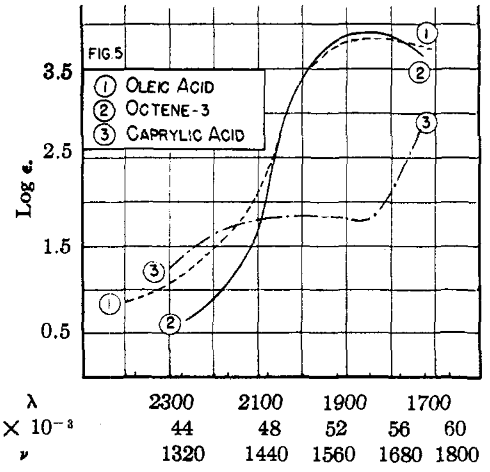

Fig. 5.—Absorption spectra of oleic acid, octene-3 and caprylic acid.

recrystallized fourteen times. The iodine number (Wijs) was 269 (theory 273.6). The absorption curve for this acid shows maxima at about 1950 Å. and 1750 Å. (Fig. 4).

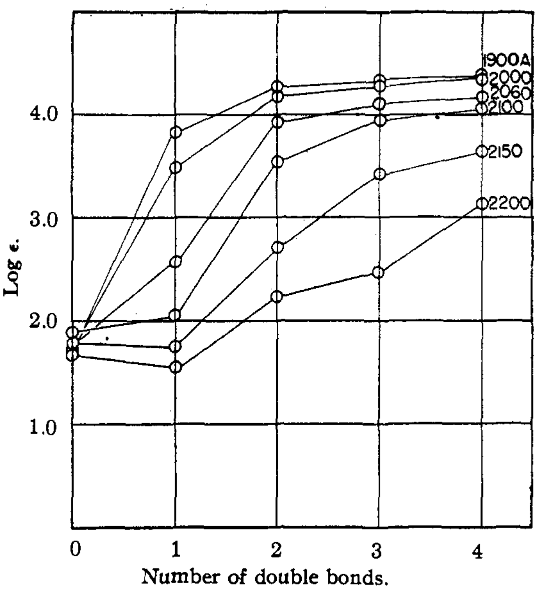

Fig. 6.—The relationship between the number of double bonds and extinction coefficients at several wave lengths for single fatty acids. The experimental points are connected by lines to aid the reader in following the absorption at each wave length. Mixtures of two fatty acids differing by one double bond would give the intermediate values on the straight line between them, but an oil averaging one double bond by having an equal amount of saturated acid and linoleic acid would not have the absorption of oleic acid glyceride.

A sample of methyl arachidonate which had been prepared by the low temperature crystallization method[18] followed by fractional distillation was also available for this study. By calculation from iodine number, this preparation was 95% pure (iodine number 294). As much as 5% of conjugated dienes, as shown by the absorption at 2350 Å. (Fig. 4), may have been produced in this acid during saponification and distillation.[19,20,21] That this band is not characteristic of natural arachidonic acid itself is shown by the much lower values reported by Barnes, *et al.*,[8] and by Mowry, *et al.*[18] The former workers found a log ε of 1.95 (2350 Å.) for methyl arachidonate in 95% ethyl alcohol; the latter, a log ε of 2.74 for arachidonic acid in absolute alcohol. The curve of Barnes, *et al.*, falls steadily with only the slightest hint of a band at 2350 Å. Although a trace of conjugated material has a marked and

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(18) D. T. Mowry, W. R. Brode and J. B. Brown, *J. Biol. Chem.*, **142**, 671 (1942).

(19) T. Moore, *Biochem. J.*, **31**, 138 (1937).

(20) F. A. Norris, I. I. Rusoff, E. S. Miller and G. O. Burr, *J. Biol. Chem.*, **139**, 199 (1941).

(21) F. A. Norris, I. I. Rusoff, E. S. Miller and G. O. Burr, *ibid.*, **147**, 273 (1943).

readily detectable effect on the curves of the unconjugated fatty acids at 2300 Å., it would scarcely alter the height or position of the maxima between 1700 and 2000 Å. because in this region the extinction coefficients of conjugated acids are about 10% of the values for unconjugated acids (compare conjugated linoleic acid in Fig. 10 with linoleic acid in Fig. 4).

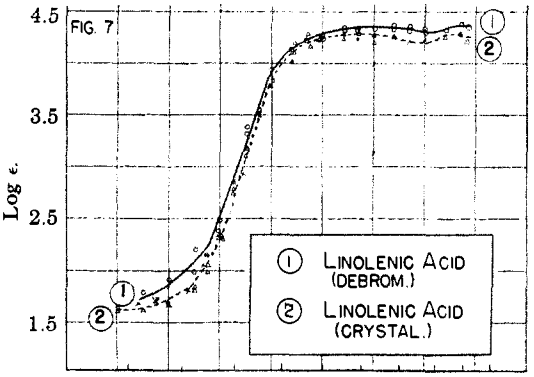

Fig. 7.—Absorption spectra of two samples of linolenic acids prepared by the method of debromination and by low temperature recrystallization.

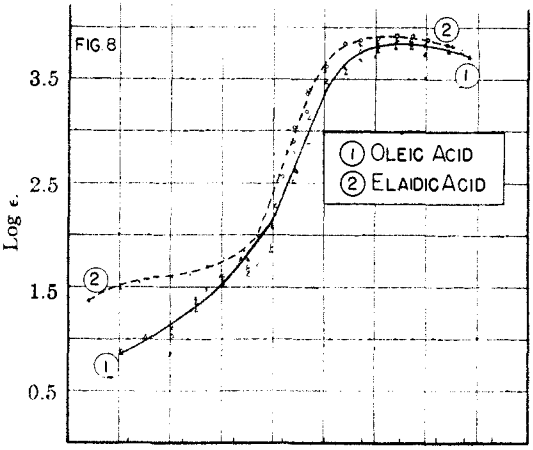

Fig. 8.—Absorption spectra of oleic and elaidic acids.

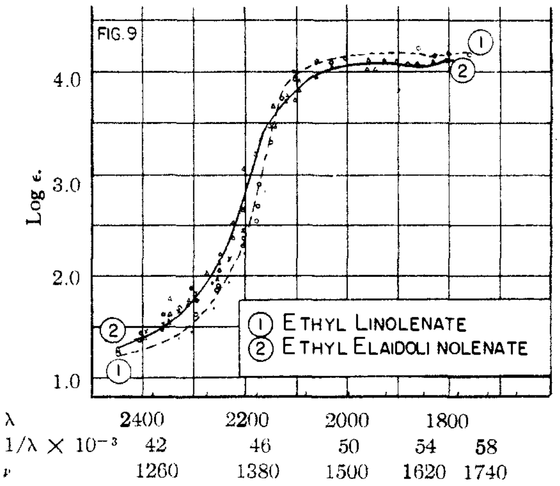

Fig. 9.—Absorption spectra of ethyl linolenate and ethyl elaidolinolenate.

Unsaturation in the fatty acids produces absorption essentially like that of unsaturated hydrocarbons. The general shape of the curve for linoleic acid is not unlike that of diallyl in the vapor phase given by Carr and Stücklen[5] although the maxima differ somewhat in height and position. It is likely that these differences are due to the effects of the solvent since octene-3 and oleic acid give almost identical maxima (1830 Å.) when measured under the same conditions in this Laboratory (Fig. 5). The divergence of the curves in the longer wave lengths may be attributed to the carboxyl group of the oleic acid which raises its absorption close to that of the saturated acids.

Figure 6 shows the relationship between the number of double bonds and the extinction coefficient at various wave lengths. The irregularities in the curve at 2200 Å. are undoubtedly due to impurities which absorb strongly at this wave length. The values at 2100 Å. are in very close agreement with those given by Barnes, *et al.*[8] It is clear that at this or shorter wave lengths absorption is greatly affected by the degree of unsaturation and becomes so intense that impurities have only minor effects. If the measurements can be made with sufficient accuracy the constants can be used for calculation of oil composition.

The Effect of Method of Preparation upon the Spectral Curves of Unsaturated Fatty Acids.—In Fig. 7 are given the curves of two samples of linolenic acid, one prepared by low temperature crystallization as described above and the other by debromination of the hexabromide from linseed oil. The latter had an iodine number of 265. The two absorption maxima of this sample occur at about 1860 and 1730 Å.

It is clear that the samples differ appreciably. Possibly debromination causes a certain amount of isomerization. It is known that *trans* isomers differ from the *cis* forms in absorption. β-Eleostearic acid, a geometric isomer of α-eleostearic, has its absorption maxima shifted about 30 Å. toward the shorter wave lengths and its maximum extinction coefficient increased about 20% over that of the α-form.

The absorption at 2300 Å. is so low that not more than a trace of conjugated material could be present in either of these samples of linolenic acid.

Effect of Geometric Isomerism on the Absorption Spectra of Unconjugated Fatty Acids.—Pure elaidic acid was prepared from oleic acid by Poutet's method.[15] Melting point determinations gave a value of 51.5° (recorded 51.58°). The effect of elaidinization of oleic acid upon spectral absorption is shown in Fig. 8. The absorption curve for elaidic acid is shifted approximately 40 Å. toward the visible end of the spectrum and the maximum absorption is increased about 15%. The increased absorption at 2350 Å. is probably due to a trace of conjugated acid.

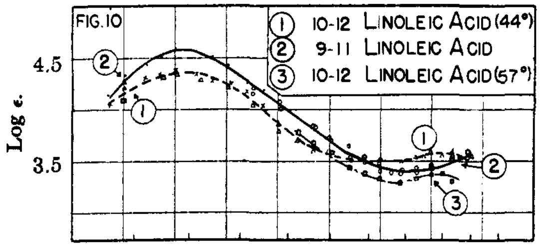

Fig. 10.—Absorption spectra of three isomeric conjugated linoleic acids.

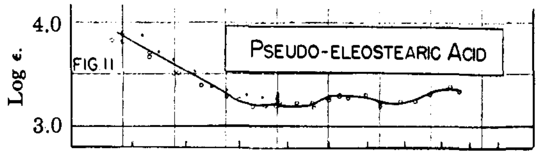

Fig. 11.—Absorption spectra of pseudo-eleostearic acid.

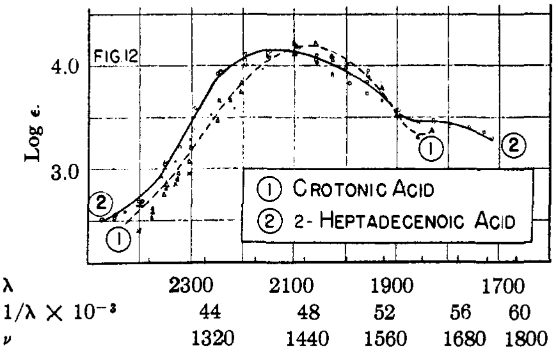

Fig. 12.—Absorption spectra of crotonic acid and 2-heptadecenoic acid.

Ethyl linolenate (iodine number 240, theory 248) was prepared by debromination of the hexabromostearic acid obtained from linseed oil. Ethyl elaidolinolenate was prepared from ethyl linolenate according to the method of Kass, Nichols and Burr[22] (iodine number 244, theory 248). The absorption curves in Fig. 9 show that the elaidinized product is shifted 25 Å. toward the visible end of the spectrum and the maximum absorption is decreased about 11%. Van der Hulst[23] has found that at the longer wave lengths (2600–2300 Å.) the absorption curve for the elaidic acid rises more slowly than that of methyl oleate. However, the data for these two compounds cannot be compared with those presented in this study since the absorption by oleic acid at 2400 Å. is much higher than was found by us. Dingwall and Thompson[24] have reported that the absorption maxima of β-eleostearic acid are about 30 Å,

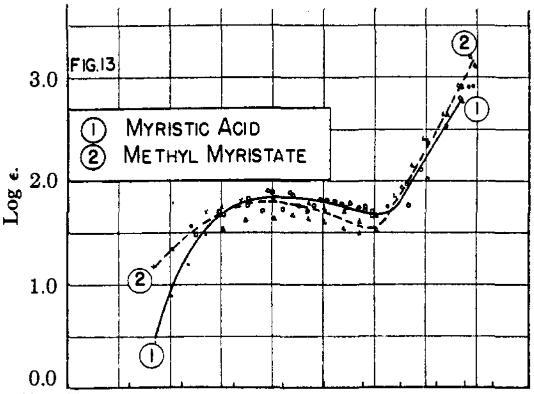

Fig. 13.—Absorption spectra of myristic acid and its methyl ester.

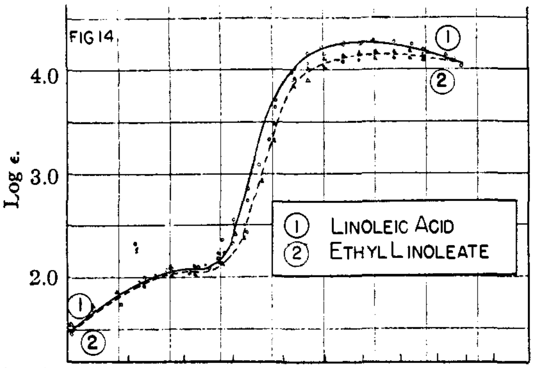

Fig. 14.—Absorption spectra of linoleic acid and its methyl ester.

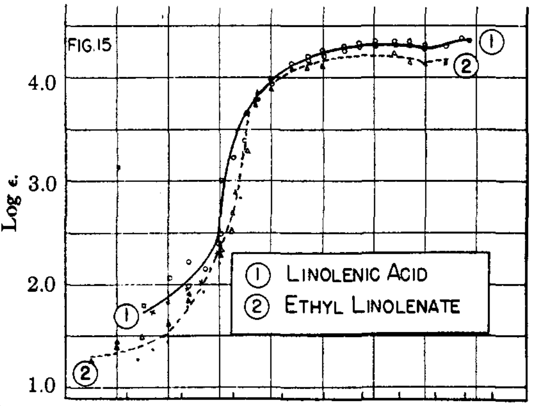

Fig. 15.—Absorption spectra of linolenic acid and its methyl ester.

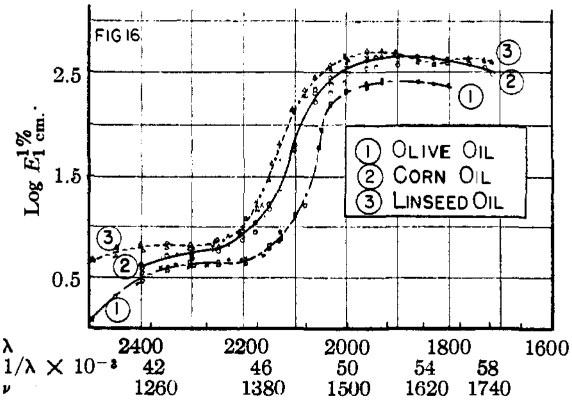

Fig. 16.—Absorption spectra of some vegetable oils.

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(22) J. P. Kass, J. Nichols and G. O. Burr, THIS JOURNAL, 63, 1060 (1941).

(23) L. J. N. van der Hulst, *Rec. trav. chim.*, 54, 639 (1935).

(24) A. Dingwall and J. C. Thompson, THIS JOURNAL, 56, 899 (1934).

toward the ultraviolet as compared with the α-form.

Effect of Isomerism on the Absorption Spectra of Conjugated Fatty Acids.—10,12-Linoleic acid (melting point 44°) was prepared according to previously described methods.[25] 9,11-Linoleic acid, melting point 54°, was prepared from castor oil according to the method of Mangold.[26] 10,12-Linoleic acid (melting point 57°) was prepared from dehydrated castor oil by the method of Kass[27] and of von Mikusch.[26] The spectral curves in Fig. 10 show that there are some differences in light absorption by these acids. The absorptions at 2300 Å. agree very well with the data in the literature. The data presented here on conjugated acids together with the work of Carr and Stücklen[5] on 1,3-pentadiene in the vapor phase show that although this chromophore absorbs strongly in the Schumann region, its highest maximum is above 2000 Å.

Pseudo-eleostearic acid (10,12,14-linolenic acid) was prepared according to the method of Kass and Burr.[28] Figure 11 shows the absorption curve for the acid. The extinction coefficients at the longer wave lengths (2500–2100 Å.) are somewhat lower than the published values for eleostearic acid.[7] No maxima were found in the Schumann region which approach the high absorption at about 2700 Å.

Light Absorption by α,β-Unsaturated Acids.—Crotonic acid was recrystallized until it had a melting point of 72°. The absorption maximum is at about 2080 Å.

2-Heptadecenoic acid was prepared according to the method of Lauer and co-workers.[29] Its absorption maximum is at about 2130 Å. Figure 12 compares these two conjugated compounds. The curve for crotonic acid agrees very well with those reported in the literature.[30] However, the extinction coefficients for 2-heptadecenoic acid are lower than those obtained by Lauer, *et al.*[29]

Effect of Esterification on Light Absorption.—The methyl and ethyl esters of the fatty acids were prepared and studied. Figures 13, 14 and 15 show the comparison between three free fatty acids and their esters. The only consistent effect of esterification is a slight drop in maximum extinction coefficient.

Absorption Spectra of Oils.—Olive oil, corn oil (Mazola) and linseed oil were studied spectroscopically and their absorption curves are given in Fig. 16. The effect of fatty acid composition is clearly reflected in the intensity of absorption at all wave lengths. However, these measurements are not of sufficient accuracy to be used as constants in simultaneous equations for the calculation of composition. The effect of double bonds on light absorption at about 2100 Å. (Fig. 6) is of such magnitude that if extinction coefficients are measured with an accuracy of one per cent. this constant is very useful in calculating the fatty acid composition of oils[8] without the use of alkali conjugation preceding the spectrographic measurement.[31,32,33]

Summary

A study of naturally occurring fatty acids and their isomers and derivatives shows the large effects of the number and position of double bonds on absorption spectra.

Geometric isomerism and esterification exert smaller effects.

The absorption of light by oils at very short wave lengths is directly dependent upon the fatty acid composition of the oil.

MINNEAPOLIS, MINN. RECEIVED NOVEMBER 20, 1944

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(25) J. P. Kass, E. S. Miller and G. O. Burr, THIS JOURNAL, **61**, 482 (1939).

(26) J. D. von Mikusch, *ibid.*, **64**, 1580 (1942).

(27) J. P. Kass, American Chemical Society Abstracts, Memphis, 1942, Section N, p. 1.

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

(29) W. M. Lauer, W. J. Gensler and E. S. Miller, *ibid.*, **63**, 1153 (1941).

(30) K. W. Hausser and A. Smakula, *Angew. Chem.*, **47**, 657 (1934).

(31) J. P. Kass, E. S. Miller, M. Hendrickson and G. O. Burr, Abstracts of Papers, 99th Meeting, American Chemical Society, Cincinnati, Ohio, April 1940, page A11.

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(33) J. H. Mitchell, Jr., H. R. Kraybill and F. P. Zscheile, *Ind. Eng. Chem., Anal. Ed.*, **15**, 1 (1943).