third double bond of the β-isomer is *trans* C=C whereas in the α-isomer it is *cis*. This has also been observed in the spectra of the maleic anhydride adducts of α- and β-elaeostearic acids by Bickford *et al.* (3). The intense bands at 10.45 μ and 10.6 μ have been attributed by them to the ring vibrations of the cyclohexene or the maleic anhydride ring or to both.

The maleic anhydride adducts of α- and β-kamlolenic acids were treated with alkaline potassium permanganate by Gupta, Sharma, and Aggarwal (6), and azelaic acid with small quantities of suberic acid was isolated from the oxidation products in both the cases. This shows that the adduct formation in the α- and β-isomers takes place at the 11,13 conjugated double bonds while the exocyclic double bond is at the 9-position, which evidently is *cis* in the former and *trans* in the latter cases.

As all three conjugated double bonds in β-kamlolenic acids are *trans,* two maleic anhydride adducts would be predicted, as in the case of β-elaeostearic acid (3), rather than only one. It has been mentioned above that the product was obtained as a thick viscous liquid and even if two adducts had been formed from β-kamlolenic acid, they could not have been separated. When the β-kamlolenic acid adduct was treated with perbenzoic acid in chloroform solution (3), no precipitate was obtained even after keeping for 48 hrs. at 0°C., showing thereby that, similar to the α-acid, only one maleic anhydride adduct is formed from β-kamlolenic acid. Further support for this conclusion is drawn from the permanganate oxidation products of the adduct from β-kamlolenic acid. No glutaric acid could be isolated, and formation of azelaic acid in sufficient yield indicates that the maleic anhydride adduct formation most probably takes place in the case of β-isomer at the 11,13-conjugated double bonds only. Hence the three double bonds in α-kamlolenic acid are *cis* 9-*trans* 11- *trans* 13 and in the β-isomer *trans* 9-*trans* 11-*trans* 13.

Summary

The *cis-trans* and positional configuration of α- and β-kamlolenic acids have been investigated. Infrared data of the two isomers, their acetyl derivatives and maleic anhydride adducts, and the permanganate oxidation products of the addition compounds, together with the selective epoxidation of the exocyclic double bond of the maleinated derivatives, have been used to confirm the structure of the three conjugated double bonds in α-kamlolenic acid as *cis*-9-*trans* 11-*trans* 13 and β-kamlolenic acid as *trans* 9-*trans* 11-*trans* 13.

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Report of the Oil Color Committee, 1954-55

THE LAST MEETING of the Oil Color Committee was held in Minneapolis at the time of the fall convention of the American Oil Chemists' Society. It was decided then to continue the investigation of the chlorophyll determination and to prepare a method for the determination of spectral transmission curves for joint use of the Color and Bleaching Committees. At the time and in a subsequent letter to each member comments were requested on the future program of the Color Committee. It was the consensus that the work on the chlorophyll determination should be completed and that the immediate work should be closely identified with that of the Bleaching Committee in determining the best method for measuring oil color and how the oil could be evaluated by bleaching methods.

Six samples of oil containing various amounts of chlorophyll were submitted to 17 members of the committee. Eight laboratories analyzed the samples. Three laboratories reported that they were unable to undertake the work outlined. Six laboratories made no reply whatsoever. The data obtained from the eight laboratories reporting are shown in Table 1. Laboratory No. 3 was not included in the average shown since the results were not in accord with the other laboratories for some unknown reason.

It should be noted that the percentage of average deviation from the mean value is about 5% for all values above approximately .1 p.p.m. This is exceptionally good for a method which is being used for the first time. The committee therefore recommends this procedure to the Society to be placed in its books as a tentative A.O.C.S. method. A copy of the final procedure is attached.

The method for the determination of spectral transmission curves to be used in the joint work of the Color Committee and the Bleaching Committee is attached and becomes a part of this report.

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Spectral Transmission Curves

This method is to be used for the determination of the spectral transmission curves on oil samples being examined by the A.O.C.S. committees on bleaching and oil color.

Reagents. Carbon tetrachloride, spectro grade, Eastman No. S-444.

Apparatus. Spectrophotometers, Beckman DU, Cary and Beckman B, with continuous sensitivity control.

Operation. Adjust the spectrophotometer to be used carefully in accordance with the directions furnished by the manufacturer. It is important to be sure that the wavelength setting is correct and that the instrument's response at the various wavelengths is as

TABLE 1

Lab. No.	Sample No.						Instrument	Cell Size
	1	2	3	4	5	6		
1	.005	.06	.26	1.63	7.93	5.64	Beckman B	....
2	.00	.07	.21	1.58	7.29	4.81	Beckman DU	....
3	(.01)	(.11)	(.12)	(2.07)	(9.03)	(5.91)	Beckman DU	10 mm.
4	....	.05	.22	1.67	7.99	5.11	Beckman DU	10 mm.
5 A	.00	.06	.25	1.81	8.84	5.68	Beckman DU	....
B	.00	.08	.22	1.59	7.94	5.17	Beckman B	....
C	.00	.07	.23	1.49	7.07	5.06	Cary	....
6	....	....	....	1.68	8.28	5.31	Beckman B	10 mm.
7 A	.00	.07	.23	1.72	8.23	5.16	Beckman DU	....
B	.005	.05	.20	1.47	7.64	5.00	Beckman DU	....
Aver.	.001	.064	.23	1.63	7.91	5.22		
Aver. Dev.	....	.009	.015	.08	.39	.22		
% Aver. Dev.	....	14.0	6.5	4.9	5.0	4.2		
6	....	0.1	.24	1.75	5.06	4.06	Coleman	
8	.00	.04	.13	1.44	4.00	3.19	Coleman	
7	.00	.03	.28	1.90	8.2	5.4	Spectronic 20	

Eight laboratories reported results.
Three laboratories were unable to do the work.
Six laboratories did not reply.

nearly correct as can be ascertained. The instrument's response should be checked, if possible, against a filter standardized by the Bureau of Standards.

After checking the instrument as fully as possible for response and wavelength setting, set the 0 and 100% transmittance points, using a standard cell containing carbon tetrachloride as the solvent. When the Beckman B spectrophotometer is used, all measurements should be made at a slit width of 0.1 mm. The slit should be fixed and the instrument adjusted between 0 and 100% response points, using the continuous sensitivity control. It will be found necessary to vary the slit on both the Beckman DU and Cary instruments in order to make the measurements. With these two instruments however the slit opening in terms of wavelength will be comparable with those obtained on the Beckman B instrument, using the fixed slit of 0.1 mm.

Fill a second cell, which has been picked to give the same percentage transmittance as the blank when filled with carbon tetrachloride, with the oil to be examined. The oil should be clear and brilliant. If not, the temperature should be raised to a point at which no solid material is present and filtered, using approximately 0.5% by weight of filter aid. Adjust the temperature of the oil in the cell to 85 ± 5°F.

Place the cell containing the oil in the instrument and read the transmittance at every 10 mμ throughout the visible range (360 to 700 mμ). Use the maximum cell length possible to give transmittance percentages between 20 and 50% (0.3 to 0.8 absorbance). It may be necessary to go to considerably higher transmittances on light-colored samples. When this is necessary, use the maximum size of cell that the instrument will accommodate. When sharp absorption peaks are observed, additional measurements should be made near the absorption points to insure that a precise curve of the oil transmittance can be drawn. Plot the complete spectral curve obtained, correcting all transmittances to a 10-mm. column before plotting.

To correct transmittances to a 10-mm. column, proceed as follows:

1. record the absorbance on the sample corresponding to the transmittance determined;
2. multiply the absorbance by $\frac{10}{\text{cell length}}$ used; and
3. read the transmittance on the instrument dial corresponding to the calculated absorbance (2).

Reporting Data. All results reported should include a spectral transmittance curve plotted at a 10-mm. column length and the data actually determined, including actual absorbance values and length of column measured.

Parts Per Million Chlorophyll

Scope. This method is used to determine p.p.m. of chlorophyll on refined and on refined and bleached oils. Parts per million of chlorophyll are calculated from measurements made on a spectrophotometer at 630, 670, and 710 millimicrons. The method is not applicable to hydrogenated oils and finished products, since the chlorophyll absorption does not occur at 670 millimicrons in processed oils.

Reagents. CCl_4, redistilled carbon tetrachloride. The transmittance should not differ from that of distilled water by more than 0.5% at 400 millimicrons.

Apparatus. Beckman B spectrophotometer, with red phototube modified with a continuous sensitivity control; Beckman DU spectrophotometer; and Cary spectrophotometer.

Sample cells, water white, 50 mm. long, 20 mm. wide, 40 mm. deep. Obtained from Pyrocell Manufacturing Company, 207-211 E. 84th street, New York 28, N. Y. These cells must be matched and show no greater spread than 0.5% transmission when checked with distilled water at 400 millimicrons. Other matched cells may be used.

Adjustment of the Spectrophotometer. Before using, the spectrophotometer should be checked carefully, following the instructions given in the operating manual obtained with the instrument. The wavelength setting of the instrument should be checked with the blue sensitive phototube in the instrument. With the instrument turned on and with no cells in the cell holder, the maximum sensitivity obtainable with the instrument should occur at approximately 540 millimicrons. It is advisable to check the instrument occasionally against a mercury vapor lamp, using 579.1-, 546.1-, 435.8-, and 404.7-millimicron checking points. Any mercury vapor lamp can be focused to pass through the instrument in place of the regular incandescent lamp normally used. Transmission peaks should occur at the specified wavelengths.

Operation. Install and adjust the instrument, following the instructions given in the manual. Wavelength checks should be made as indicated above and the carbon tetrachloride should be checked against

distilled water. Turn on the instrument at least 15 min. before use.

Place a 50-mm. cell containing the CCl_4 in the cell compartment. Set the instrument to the desired wavelength, using the wavelength knob. Turn the sensitivity knob to position "1" and, with the cell aperture closed, adjust the dark current to give a reading of zero. Open the cell aperture and adjust the slit to 0.1 mm. With the continuous sensitivity control adjust the reading to 100%. Again check the dark current and readjust if necessary. Close the cell aperture. Fill a second cell with the sample, which must be clear and brilliant. Adjust the temperature of the oil to 85°F. ±5°F. Place in the instrument.

Open the cell aperture and read the absorption as shown by the meter. Close the cell aperture and read the absorption as shown by the meter. Close the cell aperture and change the wavelength setting to the next desired wavelength with CCl_4 in the light beam. Readjust the dark current and sensitivity control to give 0 and 100% readings. Again put the sample in the light beam. Make all of the desired readings following these procedures.

All absorbance values should fall between 0.3 and 0.8 except on the Cary instrument, where values up to 3.0 are acceptable. Use the maximum cell size to give the desired values.

Calculations

p.p.m. chlorophyll, using Beckman "DU" =

$$\frac{A_{670} - \frac{A_{630} + A_{710}}{2}}{0.1016\ L}$$

p.p.m. chlorophyll, using Beckman B with red sensitive tube =

$$\frac{A_{670} - \frac{A_{630} + A_{710}}{2}}{0.0964\ L}$$

p.p.m. chlorophyll, using Cary =

$$\frac{A_{670} - \frac{A_{630} + A_{710}}{2}}{.1086\ L}$$

L = length in cms.

This method of determining chlorophyll is not applicable to hydrogenated oils, deodorized oils, and finished products since the chlorophyll absorption no longer occurs at 670 millimicrons.

The Coleman Jr. Spectrophotometer can be used for determining chlorophyll in amounts above 0.1 p.p.m. Readings are made in the 25-mm. cuvette against CCl_4 at 630 and 670 millimicrons.

$$\text{p.p.m. chlorophyll} = \frac{A_{670} - A_{630}}{0.0668}$$

Products of the Lipoxidase-Catalyzed Oxidation of Sodium Linoleate[1]

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ALTHOUGH considerable progress has been made in elucidating the mechanism whereby soybean lipoxidase catalyzes the oxidation of polyunsaturated fatty acid compounds, the reactions are sufficiently complex so that a number of details await further clarification. Bergström (2) found that the monohydroxystearic acids and other hydroxylated compounds present in the hydrogenated products from autoxidation and lipoxidase-catalyzed oxidation of linoleate were quite similar. On the basis of this and other evidence he deduced that the principal products of the lipoxidase-catalyzed oxidation of linoleate were 9 and 13 hydroperoxides.

Subsequent studies led to the belief by some investigators (1, 8) that the lipoxidase-catalyzed and autoxidation reaction mechanisms were both chain reactions, the lipoxidase serving primarily to create free radicals for the chain mechanism. However Tappel *et al.* (15, 16) cast doubt on the role of lipoxidase as an initiator of reaction chains of the autoxidative type and reported that the lipoxidase oxidation exhibited many of the characteristics of ordinary types of enzyme reactions.

Earlier studies by Bergström and Holman (3) have led to an apparent molecular extinction coefficient of 31,400 for the products of the lipoxidase oxidation of sodium linoleate at 0°C. with a pure lipoxidase preparation. Since this was considerably higher than the molecular extinction coefficient observed for the products of autoxidation, it appeared at that time that the lipoxidase oxidation led to a higher proportion of conjugated diene hydroperoxides (3). More recent studies (4, 11) have revealed that at least 90% of the hydroperoxides formed in the low temperature autoxidation of methyl linoleate are also conjugated dienes; the lower molecular extinction coefficient for the products of autoxidation could be attributed to the presence of predominantly *cis, trans* isomers. On this basis it might appear that the higher molecular extinction coefficient observed quite consistently for the products of lipoxidase-catalyzed oxidation could be attributed to the formation of *trans, trans* conjugation (or possibly *cis, cis* conjugation since the molecular extinction coefficient of the latter has not been determined). However, although crude lipoxidase preparations were used in our work, this possibility appeared to be very unlikely in view of the results of the present investigation in which it was found that the diene conjugation existed predominantly in the *cis,trans* configuration when the oxidation was conducted under mild conditions. Presented herewith is a detailed study of the products formed in the lipoxidase-catalyzed oxidations conducted under relatively mild conditions to obtain more information on the nature of the reaction.

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