acids under specified conditions. With the approval of the committee, certain additions have been made to the apparatus specifications, and a requirement is made that color be determined by the A.O.C.S. Tentative Method Ka 3-58, using 1953 Gardner Color Standards. This instruction is of a temporary nature until the committee can determine and advise their preferred method for color measurement. All development work on this method was performed by use of the Gardner Color Standards.

The Uniform Methods Committee approves and recommends adoption of this method as "Tentative." *Adopted.*

3) *Titer Test, L 6a-55*

Action upon a proposed revision of this method was deferred until the annual meeting next spring in order to permit more time for incorporation into it of certain items of apparatus which are dependent upon the adoption of the proposed revision of Cc 12-41, as recommended above. This deferment will not delay the issue of this method in printed form so it will be included with the 1949 revisions.

The Statistical Committee, W. E. Link, chairman, has been active in carrying out the requests made of it by the Uniform Methods Committee following the last annual meeting. By the end of this year we hope to have a standard method for evaluating the precision of any analytical method, and a standard format for expressing it. A number of committees have submitted such data, but their addition to the appropriate methods is being delayed temporarily in order to bring them within the desired uniformity of expression.

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Ricinelaidic Acid and Methyl Ricinelaidate. Their Preparation and Determination by Infrared Spectroscopy

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IN CONNECTION with other work in progress at this laboratory, an analytical procedure for the determination of ricinelaidic acid (*trans*-12-hydroxy-9-octadecenoic acid) and its methyl ester in elaidinization mixtures was required, and, for this, authentic samples of these pure compounds were necessary. Infrared absorption procedures for the determination of *trans* isomers (8, 18, 20) have proved satisfactory for elaidic acid and methyl elaidate, but characteristics of the spectra of ricinelaidic acid and methyl ricinelaidate necessitated modifications of the equations.

The purpose of this paper is to present a simplified method for the satisfactory preparation of ricinelaidic acid and its methyl ester, a procedure for their determination by means of infrared absorption spectra and data concerning the composition of the equilibrium mixture of methyl ricinelaidate-methyl ricinoleate. Characteristic features of the infrared spectra as additional criteria of purity of the *trans* isomers and a mechanism for the elaidinization are discussed.

Experimental

Starting Materials

Castor Oil. The castor oil employed in this investigation had the following characteristics:

I.V., 85.3; sap. value, 180.1; acid value 10.4;% hydroxyl, 5.2; $[\alpha]^{23/D} + 4.46$; $n^{25/D}$ 1.4784.

Methyl Ricinoleate. This ester was prepared by alcoholysis of castor oil, followed by fractional distillation of the crude esters (19). The methyl ricinoleate obtained had the following characteristics:

Anal.: calcd. for $C_{19}H_{36}O_3$: C, 73.02; H, 11.61; % hydroxyl, 5.4; sap. equiv., 312.5; I.V., 81.2.
Found: C, 72.69; H, 11.65; % hydroxyl, 5.2; sap. equiv., 312.0; I.V., 81.8; $[\alpha]^{25/D} + 5.00$; $n^{25/D}$ 1.4609.

Ricinoleic Acid. Ricinoleic acid was obtained through saponification of methyl ricinoleate, followed by low-temperature crystallization of the recovered acid (4). The acid had the following characteristics:

Anal.: calcd. for $C_{18}H_{34}O_3$: C, 72.43; H, 11.48; % hydroxyl, 5.7; I.V., 85.0.
Found: C, 72.18; H, 11.39; % hydroxyl, 5.8; I.V., 84.8; $n^{25/D}$ 1.4710.

Preparation of Methyl Ricinelaidate

The *cis* to *trans* isomerization of the ethylenic bond of methyl ricinoleate was effected in the following manner. To 150 g. of methyl ricinoleate, maintained at 60°C., were added with rapid stirring 10 ml. of 2M sodium nitrite solution and 6.66 ml. of 6M nitric acid. The heating and stirring were continued for 3 hrs., after which time the reaction mixture was poured into 500 ml. of water contained in a separatory funnel. The elaidinized esters were extracted with ether, washed with distilled water until free of acid, and finally dried over sodium sulfate. The product recovered from the solvent weighed 142 g. and contained *circa* 76% methyl ricinelaidate as determined spectrophotometrically. The methyl esters were dissolved in 690 ml. of pentane-acetone (5:1) and allowed to crystallize at −20°C. over-night. After filtration, the crystals were washed with 100 ml. of cold pentane and dried *in vacuo*, yield 82.9 g. This material was dissolved in 500 ml. of acetone-water (9:1), treated with a decolorizing carbon, filtered, and allowed to crystallize at −20°C. over-night. The solution was filtered, and the crystals were washed with 50 ml. of cold acetone. After drying *in vacuo*, there were obtained 66.5 g. of methyl ricinelaidate, m.p. 28.9–29.8°C.

Anal.: calcd. for $C_{19}H_{36}O_3$: % hydroxyl, 5.4; sap. equiv., 312.5; I.V., 81.2.
Found: % hydroxyl, 5.7; sap. equiv., 312.8; I.V., 81.4 $[\alpha]^{23.4/D} + 4.35$ (35% solution in acetone); $n^{60/D}$ 1.4470; absorptivity, a = 0.459 at 10.28μ in CS_2.

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Preparation of Ricinelaidic Acid

A 50.0-g. sample of methyl ricinelaidate was saponified by refluxing with alcoholic potassium hydroxide (15 g. of potassium hydroxide, 12.5 ml. of water, and 125 ml. of 95% ethanol) for 30 min., swirling the flask occasionally during that period. After cooling, the soap was acidified in a separatory funnel with 200 ml. of 2N hydrochloric acid. The aqueous phase was separated, and the acids were extracted with ether, washed with water, and dried over sodium sulfate. After removal of the solvent, there were obtained 44.2 g. of a product which, on crystallization from 270 ml. of acetone-water (9:1) at −20°C., yielded 33.1 g. of ricinelaidic acid, m.p. 50.5–51.1°C.

Anal.: calcd. for $C_{18}H_{34}O_3$: % hydroxyl, 5.7; neut. equiv., 298.4; I.V., 85.0.
Found: % hydroxyl, 5.7; neut. equiv., 299.4; I.V., 84.8. $[\alpha]^{23.7/D}$ + 4.54 (24% solution in acetone); $n^{60/D}$ 1.4568; absorptivity, a = 0.537 at 10.28 μ in CS_2.

Preparation of Primary Standards for Infrared Measurements

In order to make certain that the products used for the infrared measurements were of the highest attainable purity, the following procedures were employed. The ricinelaidic acid was twice recrystallized from five volumes of pentane-acetone (5:1) at 5°C., m.p. 51.0–51.4°C., and finally from 10 volumes of carbon disulfide at the same temperature, m.p. 51.0–51.5°C.

Anal.: calcd. for $C_{18}H_{34}O_3$: C, 72.43; H, 11.48; % hydroxyl, 5.7; neut. equiv. 298.4.
Found: C, 72.38; H, 11.63; % hydroxyl, 5.7; neut. equiv. 298.1. $n^{60/D}$ 1.4568; absorptivity, a = 0.558 at 10.27 μ in CS_2.

The methyl ester was then prepared by treating a sample of ricinelaidic acid with an excess of diazomethane in ether. The recovered ester was crystallized from three volumes of pentane-acetone (5:1) at −5°C., m.p. 29.6–30.4°C.

Anal.: calcd. for $C_{19}H_{36}O_3$: C, 73.02; H, 11.61; % hydroxyl, 5.4; sap. equiv., 312.5.
Found: C, 72.67; H, 11.72; % hydroxyl, 5.7; sap. equiv., 312.6; $n^{60/D}$ 1.4468; absorptivity, a = 0.482 at 10.27 μ in Cs_2.

Infrared Spectra and Quantitative Determination

Infrared absorption curves of ricinelaidic acid and methyl ricinelaidate were obtained with a Perkin-Elmer Model 21 infrared spectrophotometer. The settings used were: resolution, 927; suppression, 3; gain, 6; response, 1; and speed, 4. Both spectra were obtained as chloroform solutions at concentrations of about 20 g./l. with an absorption cell 0.50 mm. in path length. Pure dry chloroform was placed in the reference beam; absorption of chloroform was completely eliminated from the resulting spectra by this differential technique. The absorption curves are shown in Figure 1.

Ahlers *et al.* (1) included the spectrum of ricinelaidic acid from 2.5 to 4.5 μ and from 5.5 to 7.5 μ in CCl_4 and from 7.5 to 15.0 μ in CS_2 in their discussion of a comparison of the infrared spectra of *cis* and *trans* isomers of C_{18} acids. The spectrum of methyl ricinelaidate has not heretofore been published to the knowledge of the authors. Both curves are shown in Figure 1 to afford a comparison of the complete spectra from 2 to 12 μ. These spectra exhibit the features attributed to saturated long-chain fatty acids and their esters (13) with the addition of the band of moderate intensity at 10.23 μ, attributed to a C—H deformation about a *trans* C=C (17). The wavelength position of maximum absorption is somewhat shorter than that usually attributed to this vibration, *i.e.*, 10.30 μ in the spectra of elaidic acid or methyl elaidate in $CHCl_3$.

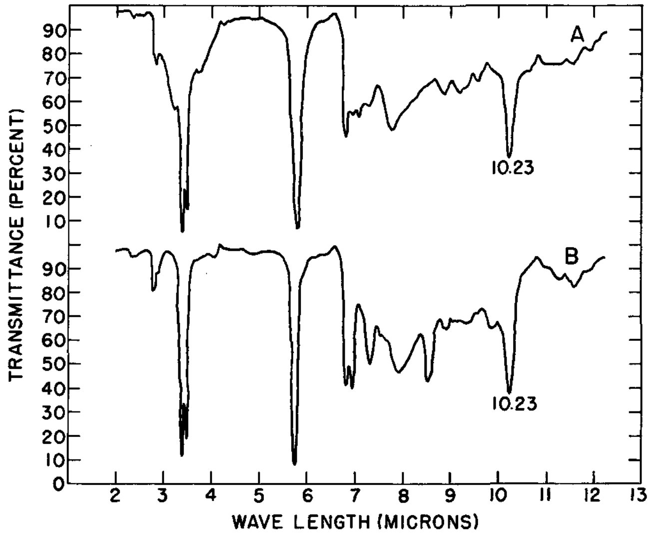

FIG. 1. Infrared spectra of A, ricinelaidic acid, and B, methyl ricinelaidate (in chloroform solution).

The band at 10.23 μ can be used for the quantitative determination of ricinelaidic acid or of its methyl ester. It has been used in this laboratory to follow the course of the elaidinization of methyl ricinoleate. The methods employed have been modifications of the procedures of Jackson and Callen (8), Shreve *et al.* (18), and Swern *et al.* (20). As most of the work in this laboratory has dealt with the analysis of the methyl esters, this procedure will be described first.

Determination of Methyl Ricinelaidate

For analysis, the samples and primary standards were dissolved in CS_2 as this solvent has proved to be the most satisfactory for quantitative measurements. As mentioned above, the C—H deformation about the *trans* C=C group appears at a wavelength somewhat less than that exhibited by the spectrum of methyl elaidate. In CS_2 the wavelength position of maximum absorption for methyl ricinelaidate is 10.27 μ, compared to 10.33 μ for methyl elaidate. The absorptivity for the methyl ricinelaidate is somewhat less than would be predicted from a comparison with methyl elaidate. For methyl elaidate, under the conditions for measurement described below, this constant has been established as 0.540. Under exactly identical conditions of measurement, the best samples of methyl ricinelaidate have an absorptivity of only 0.482. Presumably these effects, both frequency shifts of the position of maximum and the intensity at the maximum, arise from some influence of the O—H group in the ricinelaidate moiety.

In the actual procedure used, the Perkin-Elmer spectrophotometer is set with the slit width control on manual as follows: slit width, 151 μ; resolution, 79; gain, 5; response, 1; suppression, 0; and speed, 2.7. The amplifier balance is set to give a definite upscale drift to the extent that the pen will move from 50% to 100% in 50 ± 3 seconds with both light beams totally obstructed. The zero is set with nothing in either beam, and the sample and reference cells are filled with CS_2 and mounted in the spectrophotometer. The optical balance is adjusted to about 95% transmission. When the optical balance shows no change over a period of 5 min., the setting is considered fixed. A record is made of the zero with the cells in place and the wavelength setting at 10.27 μ. The 100% is determined by scanning the CS_2 in triplicate between 9 and 11 μ. It is only necessary to determine the 100% line once in a day's operation. The scanning procedure is repeated with the sample solution in the sample cell. The base-line corrected absorbance,

$$A = \text{Log}\frac{\%\ \text{Transmission at Maximum}}{\%\ \text{Transmission at Base-line}},$$

is obtained by the base-line technique of Wright (21) and of Heigl *et al.* (7). The base-line is drawn from 10.02 to 10.59 μ. The percentage of *trans* isomer, as methyl ricinelaidate, is calculated from the equation:

$$\%\ trans = \frac{A}{0.374\ bc} \times 100$$

where b is the exact path length of the cell in centimeters, c is the concentration of the CS_2 solution in grams per liter, and 0.374 is the base-line corrected absorptivity for the methyl ricinelaidate, when measured under the specific conditions outlined.

As the O—H group in this molecule appears to be affecting the intensity, it seemed advisable to check the applicability of the Beer-Lambert Law. A series of CS_2 solutions of the methyl ricinelaidate primary standard of different concentrations was measured, and the absorbances at the 10.27 μ maximum were plotted against the known concentrations. The results (Figure 2) show that the Beer-Lambert Law is applicable over a rather wide range of concentrations. The method was applied to the determination of methyl ricinelaidate in elaidinized mixtures of methyl ricinoleate. The repeatability in duplicate analyses and the accuracy obtainable by the method are illustrated by the results for the analysis of known mixtures of methyl ricinelaidate and methyl ricinoleate (Table I). Results for a typical elaidinization experiment are shown in Table II. For satisfactory results all measurements of transmission should be within the limits 20 to 70%. Calculated absorptivities from duplicate analyses should check within ± 3% of the values obtained.

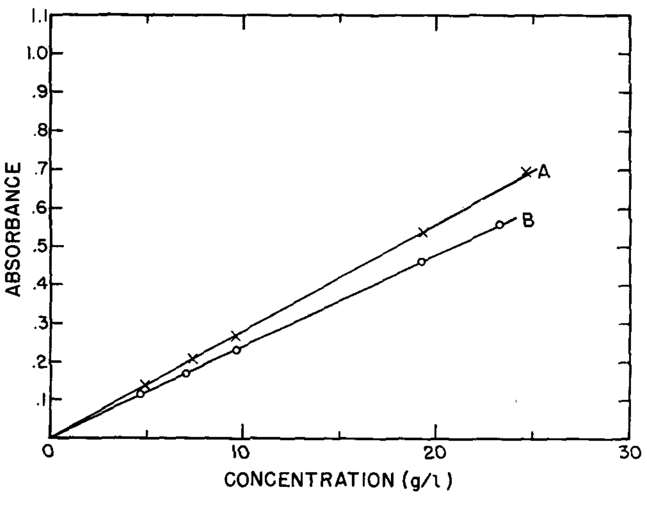

FIG. 2. Conformity to Beer's Law at 10.27 μ of A, ricinelaidic acid, and B, methyl ricinelaidate (in carbon disulfide solution).

TABLE I

Tests of Repeatability and Accuracy of Infrared Absorption Method for Methyl Ricinelaidate-Methyl Ricinoleate Mixtures

Sample No.	Absorptivity at 10.27 μ	Absorptivity baseline corrected	Methyl ricinelaidate found (%)	Methyl ricinelaidate known (%)
1	0.086 0.089 Av. 0.088	No band No band	0 0 Av. 0	0
2	0.126 0.126 Av. 0.126	0.037 0.037 Av. 0.037	9.9 9.9 Av. 9.9	10.0
3	0.185 0.186 Av. 0.186	0.093 0.092 Av. 0.092	24.9 24.6 Av. 24.8	24.9
4	0.283 0.281 Av. 0.282	0.185 0.183 Av. 0.184	49.5 48.9 Av. 49.2	50.0
5	0.390 0.382 Av. 0.386	0.285 0.278 Av. 0.282	76.2 74.3 Av. 75.2	75.1
6	0.439 0.449 Av. 0.444	0.334 0.340 Av. 0.337	89.3 90.9 Av. 90.1	89.5
7	0.475 0.481 Av. 0.478	0.369 0.372 Av. 0.370	98.7 99.5 Av. 99.1	100

Determination of Ricinelaidic Acid

In the measurement of the acid the base-line technique is difficult to apply with accuracy, owing to the interfering 10.6 μ band, characteristic of the COOH group (1). For this reason, correction for this determination has been made from measured values of ricinoleic acid. The infrared spectrum is scanned, as in the case of the methyl ester, with identical instrumental settings, but a measurement is made only at the position of maximum absorption. The percentage of *trans* isomer, as ricinelaidic acid, is obtained from the equation:

$$\%\ trans = \frac{a - 0.146}{0.558 - 0.146} \times 100$$

when a is the absorptivity of the sample at the maximum 10.27 μ; 0.146 is the absorptivity for the ricinoleic acid primary standard; and 0.558 the absorptivity for the ricinelaidic acid primary standard when measured under the conditions specified. The applicability of the Beer-Lambert Law to acid solutions is shown in Figure 2.

TABLE II

Infrared Absorption Measurements of a Typical Methyl Ricinoleate Elaidinization Experiment [a]

Elaidinization time (hours)	Absorptivity at 10.27 μ [b]	Absorptivity baseline corrected [b]	*Trans* isomer (%) [b]
0.5	0.326	0.223	59.6
1	0.364	0.262	70.2
2	0.384	0.281	75.0
3	0.388	0.284	75.8
4	0.391	0.285	76.2
5	0.384	0.278	74.2
6	0.396	0.287	76.6
7	0.390	0.282	75.5
8	0.396	0.288	76.8

[a] Elaidinization carried out at 60°C., employing 5 ml. 2M $NaNO_2$ and 5 ml. 6M HNO_3 per 150 g. of methyl ricinoleate.
[b] Average of two closely agreeing values.

Discussion

The preparation of ricinelaidic acid has been reported by a number of investigators. Rankov and Iovchev (15), for instance, have made reference to 12 publications, and there are a number of others dealing with the subject (1, 2, 4, 11, 16). Practically all of the methods for the preparation of ricinelaidic acid employ either ricinoleic acid or the castor oil fatty acids as a starting material. The isomerization catalyst most frequently used was an aqueous nitrite-nitric acid mixture as described by Mangold (11) although sulfur and selenium have been employed (15, 16) to effect the *cis* to *trans* transformation.

Numerous elaidinization experiments carried out in this laboratory have shown that the large amount of aqueous catalyst employed by previous workers is not only unnecessary but may even be disadvantageous owing to the more extensive side reactions that occur. Moreover difficulties were encountered in the crystallization procedures, caused in part by the voluminous nature of the precipitate, when either crude ricinoleic acid or the mixed fatty acids of castor oil were employed as a starting material. This phenomenon may be attributed to extensive hydrogen bonding (14) among the various species of acids present, which resulted in poor yields of rather impure products. When the mixed methyl esters obtained from the alcoholysis of elaidinized castor oil were employed in the crystallization procedure, no voluminous precipitates were encountered. The yield of pure methyl ricinelaidate however, obtained in this manner, was rather low because of the mutual solubility relationships existing in such a system. All of the aforementioned difficulties were obviated by the use of pure methyl ricinoleate as a precursor, and satisfactory yields of methyl ricinelaidate were obtained after only one recrystallization. This procedure has the additional advantage of a direct preparation of methyl ricinelaidate, which has not been reported previously, from which the pure acid may be readily obtained in good yield. Under the exact conditions of measurement described, infrared absorption measurements established the following additional criteria of purity for both ricinelaidic acid and for its methyl ester:

In chloroform solution,
  Ricinelaidic acid: maximum 10.23μ, a = 0.442
  Methyl ricinelaidate: maximum 10.23 μ, a = 0.403.
In carbon disulfide solution,
  Ricinelaidic acid: maximum 10.27 μ, a = 0.558
  Methyl ricinelaidate: maximum 10.27 μ, a = 0.482.

The results obtained from a series of six elaidinization experiments carried out on pure methyl ricinoleate, in which the amounts of catalyst were varied, indicate that the *cis* to *trans* conversion of the ethylenic bond is approximately 76%. This observation is of particular interest in view of the well established equilibrium point of 67% for the oleic-elaidic transformation. The difference in the two systems undoubtedly reflects the influence of the hydroxyl group on the ethylenic bond of the ricinoleate either through electronic or steric effects or possibly both. The oleic-elaidic acid and ricinoleic-ricinelaidic acid conversions are both readily effected by oxides of nitrogen. Of the oxides, N_2O, NO, and NO_2, it is claimed that only NO_2 is an effective isomerization agent (9, 10). This also may be the case in the aqueous nitrite-nitric acid system employed for isomerization. It is of more than passing interest to note that NO_2 (as well as NO) is paramagnetic, indicating the presence of an unpaired electron (6). It is possible therefore for NO_2 to form a "π-complex" (12) involving one of the π-electrons of the double bond of the fatty acid, thereby allowing the opportunity for *cis* to *trans* isomerization. The mechanism of this reaction may be analogous to that described for the elaidinization of oleic acid employing selenium as the catalyst, in which the formation of a "π-complex" is postulated (5).

Summary

Improved methods for the preparation of ricinelaidic acid and methyl ricinelaidate are described. The methods depend upon the elaidinization of methyl ricinoleate with a relatively small quantity of a nitrite-nitric acid solution, fractional crystallization of the methyl ricinelaidate, and its subsequent hydrolysis to ricinelaidic acid. The infrared spectra of these two compounds are presented, and bands arising from a deformation of the C—H about the *trans* C=C group are discussed. Absorptivity values for this band in chloroform and in carbon disulfide solutions are given as additional criteria of purity of ricinelaidic acid and its methyl ester. The infrared absorption procedure for the quantitative determination of isolated *trans* ethylenic bond was applied to the determination of both ricinelaidic acid and methyl ricinelaidate. Details of specific procedures for the determination of each compound are given with the equations necessary for their calculation. Use of these methods is illustrated with the analysis of elaidinization mixtures. The repeatability and accuracy obtainable are given. A value is reported for the methyl ricinoleate—methyl ricinelaidate equilibrium and the mechanism of the reaction is discussed briefly.

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