oleic acid, 1:00; Nacconol N R S F,[3] 1.03. Swatches of Soap of desaturated product, 0.81; soap of technical white woolen flannel were soiled and washed according to standard procedures (loc. cit.). Relative efficiencies of the detergents used were indicated by reflectance measurements made on the swatches in the various conditions. Average reflectances found were:

Unsoiled washed cloth	0.697
Soiled cloth	0.258
Soiled cloth washed with soap of coconut-oil acids	0.583
Soiled cloth washed with soap of desaturated product	0.629

The desaturated product from dichlorinated oleic acid was esterified with pentaerythritol and with Pentek 43.[4] Films cast on glass from mixtures of thinner, Japan drier, and these esters dried hard overnight.

Summary

Catalytic vapor-phase dehydrochlorination at pressures below 1.5 mm. and at temperatures from 220° to 350° C. was applied to chlorination products of methyl palmitate, palmitic, stearic, oleic, and linoleic acids. Clear, pale desaturated products were recovered from each material.

About half the combined chlorine was readily removed from chlorinated methyl palmitate containing an average of two atoms of chlorine per molecule. The halogen that remained was strongly held by the ester but was easily removed from the chlorinated free acid regenerated from the ester by saponification.

Product recovery from chlorinated palmitic acid became less as the degree of chlorination was increased from an average of two to an average of four atoms of halogen per molecule of acid.

Oleic acid saturated with chlorine by addition gave lower yields of recovered product than did either palmitic or stearic acid chlorinated to a comparable degree. Still greater product losses were encountered in processing linoleic acid chlorinated by addition.

Losses occurring in the process were traceable almost entirely to the tendency of the desaturated products to polymerize in contact with the solid catalyst. Besides lowering the yields of recoverable product, the accumulation of non-volatile polymers on the catalyst surfaces lowers the activity of the catalyst and shortens its period of usefulness.

Tests of the desaturated product from dichlorinated palmitic acid indicated that solutions of its sodium soap are good detergents, having better than average wetting and foaming powers.

A product having considerable conjugated unsaturation was obtained from chlorinated oleic acid. When esterified with polyhydric alcohols, this material was capable of forming drying films.

Acknowledgment

We wish to express our appreciation to E. B. Kester for the interest and direction he has given to this work, to the various members of the Fats and Waxes Section, to the Mechanical Services Division for technical assistance and aid in construction of apparatus, to G. F. Bailey for spectrophotochemical measurements and calculations, and to L. M. White and G. E. Secor for several microchemical analyses necessary to the study.

REFERENCES

1. Van Atta, G. R., Houston, D. F., and Dietrich, W. C., accompanying article.
2. Brice, B. A., Swain, M. L., Schaefer, B. B., and Ault, W. C., Oil and Soap *22*, 219 (1945).
3. American Association of Textile Chemists and Colorists Yearbook. *17*, 216 (1940).

---

[3] Trade name for a commercial product reported to consist essentially of sodium dodecyl and tetradecyl benzene sulfonate.

[4] Trade name for a commercial product reported to contain about 50% pentaerythritol and 50% dipentaerythritol.

The Spectrophotometric Determination of the Alpha and Beta Isomers of Eleostearic Acid In Tung Oil*

ROBERT T. O'CONNOR and DOROTHY C. HEINZELMAN
Southern Regional Research Laboratory,[1] New Orleans, Louisiana

and

R. S. McKINNEY and F. C. PACK
Tung-Oil Laboratory[2]
Gainesville, Florida, and Bogalusa, Louisiana

STUDIES widely reported in the literature have indicated that only the *alpha* isomer of eleostearic acid is elaborated in the tung oil of the common species (1). The *beta* isomer occurs later as a result of isomerization of the *alpha* form. Many lots of commercial tung oil are subjected in storage and handling to conditions which promote this isomerization. A method for the determination of the *alpha* form of the acid in freshly extracted tung oil by use of spectrophotometric principles has been described recently (2). But as the *beta* form of the acid has a somewhat different absorption spectrum from the *alpha*, the presence of the *beta* eleostearic acid in most samples interferes with the determination of the *alpha* by this method. Hence, a procedure is desired that will permit the quantitative determination of both the *alpha* and *beta* isomers in the presence of each other and at the same time permit the determination of the total amount of eleostearic acid present.

---

* *Presented at the 20th annual fall meeting, American Oil Chemists' Society, Oct. 30-Nov. 1, 1946, in Chicago.*

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[2] One of the field laboratories under the direction of the Agricultural Chemical Research Division of the Bureau of Agricultural and Industrial Chemistry.

No chemical methods are known for the determination of the individual isomers when both forms are present in tung oils, and the development of a spectrophotometric procedure for this purpose would be a distinct advance. Dingwall and Thomson (3) have taken advantage of the slight differences in the absorption spectra of pure *alpha* and pure *beta* eleostearic acids to show how these compounds can be quantitatively determined in the presence of each other. But they did not extend their work to the actual determination of these acids in tung oil. The accuracy and applicability of their procedure are limited also somewhat by the fact that their measurements were made with a photographic instrument.

This communication describes an application of the well-known method for the spectrophotometric analysis of binary systems (4, 5, 6, 7) to the simultaneous determination of the *alpha* and *beta* isomers of eleostearic acid in tung oil and reports some tests of the reliability of the proposed method. Results are compared with those obtained by use of chemical methods for the determination of total eleostearic acid.

Preparation of the Pure Acids

The purest compounds obtainable are essential for the evaluation of extinction coefficients to be used as standards. The *alpha* isomer of eleostearic acid is unstable (2) and the spectrophotometric measurement of it must therefore be made immediately upon its preparation. The *beta* isomer can be preserved for some time under refrigeration in the dark.

The *alpha* isomer was prepared by a modification of the method described by Nicolet (8). Three hundred grams of tung oil were saponified with a mixture of 600 ml. of ethanol (95%) and 60 gm. of sodium hydroxide dissolved in 75 ml. of water, by shaking and heating on a water bath under reflux for one hour. The mixture was then shaken until it became solid. Saponification was then complete, and the fatty acids were liberated by adding 2 liters of hot water containing an excess of sulfuric acid, and heating was continued. The *alpha* isomer was separated and purified by solution and crystallization twice from ethanol (95%) and once from petroleum ether (Skellysolve F). The purified crystals were dried under vacuum at room temperature in the dark.

The *beta* isomer was prepared by two very similar methods. In the first, tung oil, containing 0.3 gm. of iodine per 100 gm., was exposed to ultraviolet light until the oil was completely solidified. Fifty grams of the solid product were melted and 100 ml. of alcoholic potash (20 gm. potassium hydroxide dissolved in 40 ml. water and 40 ml. ethanol) were added. The mixture was heated gently and agitated until the saponification was complete. The soaps were then hydrolyzed in a 1,000-ml. flask by addition of 500 ml. of hot water, followed by addition of 70 ml. of hydrochloric acid (1:1), and by heating until the reaction was complete. The fatty acids were separated, washed free of mineral acids, and dissolved in petroleum ether (Skellysolve F). The ether solution was cooled until crystals formed. The crystals were separated by filtration. The *beta* isomer was purified by successive recrystallizations from petroleum ether, a mixture of petroleum ether and acetone, and acetone. The purified crystals were dried under vacuum at room temperature.

The second method used was a modification of Morrell's (9) procedure. Tung oil was exposed to direct sunlight for several hours and cooled in a refrigerator until the solid *beta* acid glyceride began to precipitate. Enough cold acetone was added to the cool irradiated oil so that the viscosity of the unisomerized oil did not interfere with rapid filtration. The solid glyceride was collected on a Büchner funnel and washed with small portions of cold acetone with minimum passage of air through the filter. Fifty grams of the solid product were saponified by the addition of 100 ml. of alcoholic potash (20 gm. potassium hydroxide in 40 ml. of water made to 100 ml. with ethanol) and agitation. The mixture was heated only if a homogeneous mass could not be obtained otherwise. The saponified mass was cooled and the potassium salt of the *beta* acid was filtered off. The salt was washed on the filter with small portions of cold ethanol, dissolved in hot ethanol, and recrystallized from cold ethanol. The potassium salt was then dissolved in 600 ml. of hot water (heating only as much as absolutely necessary to dissolve the product) and hydrolyzed by addition of an excess of dilute hydrochloric acid (6 ml. of 36% hydrochloric acid diluted 1:1). The *beta* acid was separated, washed free of mineral acid with water, and dissolved in ethyl ether to free the product from traces of the potassium salt. After evaporation of the ethyl ether the crystalline *beta* isomer was dissolved and recrystallized twice from this solvent, and finally dried under vacuum at room temperature.

Spectrophotometric Measurements

As the successful use of the spectrophotometric method of binary analysis depends upon the determination of extinction coefficients at precisely specified wave lengths, the solvent effect on the position of characteristic absorption in the nonpolar hydrocarbon solvent, cyclohexane, as compared to the polar solvent, ethanol, would constitute a serious source of error. Therefore, extinction coefficients for the pure eleostearic acids must be obtained in solvents in which tung oil is readily soluble. Extinction coefficients for *alpha* eleostearic acid in ethanol, isooctane, and cyclohexane have been reported and discussed in a previous paper (2). Tung oil is relatively insoluble in ethanol, the solvent most commonly used in measuring the absorption properties of unsaturated acids, and *beta* eleostearic acid is but sparingly soluble in isooctane. Cyclohexane was therefore selected for the present work. Extinction coefficients for the *beta* isomer were determined in both cyclohexane and ethanol, the latter being used to permit comparisons of values with those reported in the literature.

The ethanol was purified by prolonged refluxing with zinc dust and sulfuric acid followed by a first distillation, and then a second distillation from zinc dust and potassium hydroxide. It had a transmission of approximately 85% at 2350 Å. when measured against distilled water using 1-cm. matched absorption cells. The cyclohexane was purified on a silica gel absorption column described elsewhere (10).

Absorption measurements were made with a Beckman Quartz spectrophotometer, using 1-cm. cells. Portions of the freshly prepared acids were accurately weighed with a microbalance, dissolved in the selected solvent, and diluted to approximately 0.005 gm. per liter. Optical densities were measured every 0.5 mμ from 265 to 280 mμ and the corresponding extinction coefficients calculated from the Bouger-Beer law equation in the form

Extinction Coefficient, $E^{gm./l.}_{1\,cm.}$ = Optical Density/cl. All extinction coefficients reported in this paper are given as $E^{gm./l.}_{1\,cm.}$, where the concentration, c, is expressed as grams per liter of solution, and the path length of the absorbing cell l is 1 cm. All measurements were made at the maximum sensitivity of the instrument, to permit use of the narrowest possible band widths, approximately 0.7 mμ at 268 mμ. The wave-length scale of the instrument was calibrated throughout this region by means of atomic lines from an A-H-4 mercury vapor lamp and all wave-length settings ascertained to be accurate to within ± 0.1 to 0.2 mμ. Table 1 shows the appreciable differences in the extinction coefficients and in the wave-length positions of characteristic absorption in ethanol and in cyclohexane. As pointed out in the previous paper (2) the extinction coefficient for the *alpha* isomer in ethanol agrees very well with that reported by van der Hulst (11) and by Miller and Kass (12). The extinction coefficient for the *beta* isomer in ethanol is in excellent agreement with the accepted value (13).

TABLE 1

Extinction Coefficients of Pure *Alpha and Beta* Eleostearic Acids[1]

	Solvent				
	Ethanol		Cyclohexane		
Wave length, mμ	268	270	269.0	271.5	276.5
Alpha eleostearic acid		183.4	149.5	168.6	122.5
Beta eleostearic acid	216.1		202.4	178.1	122.5

[1] The underscored values are the extinction coefficients at the highest maxima. Values at other wave lengths are additional extinction coefficients used in obtaining the simultaneous equations.

Complete absorption curves of the two pure isomers, in cyclohexane, are shown in Fig. 1, A and B. Fig. 1, C and D, shows for comparison the absorption of two tung oil samples, a fresh oil containing a relatively large proportion of the *alpha* isomer (Fig. 1 D) and an ultraviolet irradiated sample containing a relatively large proportion of the *beta* isomer (Fig. 1 C).

These curves show that the absorption differences between the two isomers are not very great. The position of maximum absorption of the *alpha* isomer occurs at a position, 271.5 mμ, at which the curve of the *beta* isomer has a very sharp slope. Similarly the position of maximum absorption of the *beta* isomer, 269.0 mμ, occurs at a position where the slope of the *alpha* curve is very steep. This means that measurements on a composite curve, as that of any mixture containing the two isomers, would of necessity have to be made at a position where the spectrum band width and the wave-length calibration of the spectrophotometer are critical. Furthermore, the wave-length position where the two curves intersect, 276.5 mμ, is located at a position where change in extinction coefficient with very slight change in wave length is great.

These observations mean that in the application of the spectrophotometric method of binary analysis, highest accuracy is probably not attainable and satisfactory accuracy can be hoped for only if the instrument used for absorption measurements is capable of isolating very narrow spectrum bands and has been calibrated carefully for accuracy of wave-length settings. All measurements described in this paper were made with a spectrum band isolation of approximately 0.7 mμ at 268 mμ and with a wave-length setting accurate to within ± 0.1 to 0.2 mμ. Tests of the accuracy are dependent upon these requirements.

The method of binary analysis by spectrophotometric measurement is well known (4, 5, 6, 7) and consists simply of making measurements at two different wave-length positions and solving two Bouger-Beer law equations simultaneously. From data in Table 1 or Fig. 1, the simultaneous equations required for the determination of *alpha* and *beta* eleostearic acids are:

$$\text{Per cent } alpha \text{ eleostearic acid} = 100\ (0.02700\ E_{271.5} - 0.02375\ E_{269.0})$$

$$\text{Per cent } beta \text{ eleostearic acid} = 100\ (0.02248\ E_{269.0} - 0.01994\ E_{271.5})$$

where $E_{269.0}$ and $E_{271.5}$ are the extinction coefficients at 269.0 and 271.5 mμ, respectively, as obtained from measurements of the tung oil samples.

The Bouger-Beer law and the data in Table 1 give directly an expression for the total eleostearic acid content of the sample:

$$\text{Per cent total eleostearic acid} = \frac{E_{276.5} \times 100}{122.5} = 0.8163\ E_{276.5}$$

With these three equations the per cent of *alpha*, *beta*, or total eleostearic acids can be obtained readily once the cyclohexane solution is prepared. A suitable concentration of tung oil is approximately 0.005 gm./l. for measurement through 1-cm. cells.

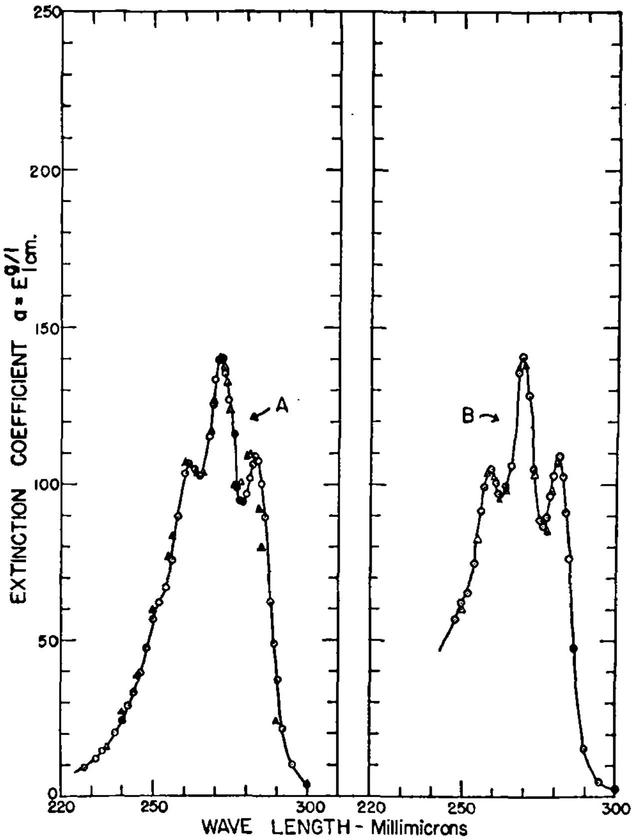

FIG. 2. Comparison of the absorption spectra of tung oil and mixtures of pure *alpha* and pure *beta* eleostearic acids in cyclohexane.

A. Fresh tung oil. B. Ultraviolet irradiated tung oil.

(Triangles ▲ represent measurement made on the tung oil sample, circles ⊙ calculations made from the per cent of *alpha* and *beta* isomers determined by the spectrophotometric method described and the absorption curves of pure *alpha* and pure *beta* eleostearic acids.)

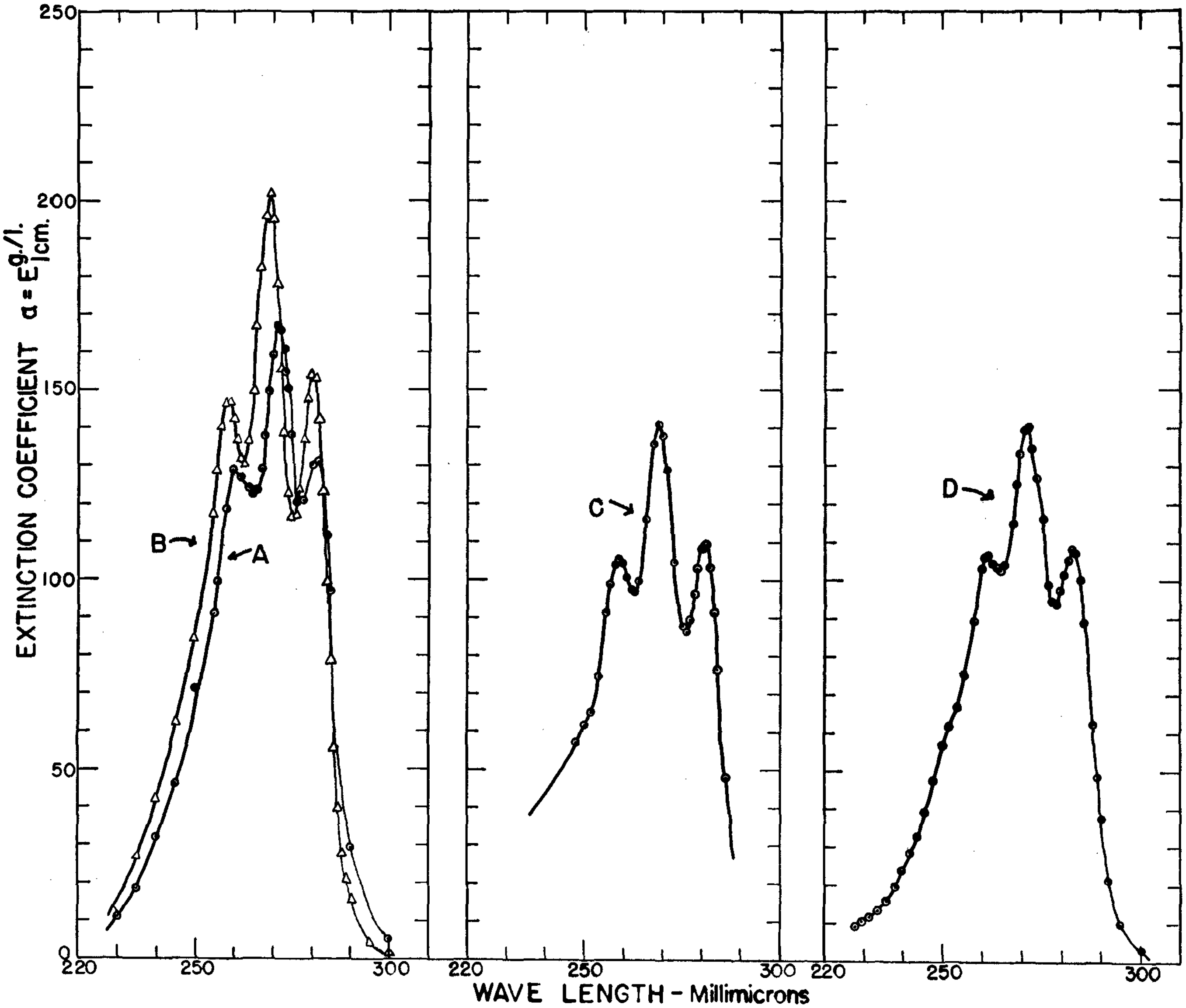

Fig. 1. Absorption spectra of eleostearic acids and tung oils in cyclohexane.

A. Pure *alpha* eleostearic acid. B. Pure beta eleostearic acid. C. Ultraviolet irradiated tung oil containing relatively large proportion (65%) *beta* eleostearic acid. D. Fresh tung oil containing relatively large proportion (80%) *alpha* eleostearic acid.

Application of the Method

Table 2 lists results obtained by the application of this method to a selected group of tung oils, including fresh oils containing relatively large proportions of alpha eleostearic acid, irradiated oils with large proportions of the *beta* isomer, stored samples of oil with intermediate amounts of both *alpha* and *beta* isomers, and oils obtained by different methods of extraction. Fig. 2 was prepared to provide some test of the accuracy of these results. If the per cent distribution of the two absorbing components of a substance (i.e., the *alpha* and *beta* eleostearic acids of tung oil) and the complete absorption curves of each are available, an absorption curve based on the analytically determined per cent composition and the value of the extinction coefficients of the pure components at each wave length throughout the spectrum can be calculated. If against such a curve the actual curve of the substance (i.e., the tung oil) is plotted, the two curves will be identical, provided the per cent compositions have been correctly distributed between the two absorbing components. The absorptions of a fresh tung oil which contains a relatively large proportion of *alpha* eleostearic acid (Fig. 2A), and of an irradiated oil containing a high proportion of *beta* acid (Fig. 2B), show the very satisfactory agreement obtained between two such curves in both cases.

A further check upon the accuracy of the procedure can be made as follows: The determined values of the *alpha* and *beta* isomers are added to give the total eleostearic acid content. This value can then be checked against an independently determined value of the total eleostearic acid content made from measurements at the wave-length position where the two curves of the pure components intersect. This is a test of the total eleostearic acid content of the oil only and not of the accuracy with which the method distributes it between the two isomeric forms. However, once the total eleostearic acid is determined, the distribution beween the two forms cannot vary appreciably if the condition of matching curves just described is to hold, for the absorption of the *beta*

TABLE 2

Analysis of Tung Oils by Chemical and Spectrophotometric Methods

Tung Oils	Chemical Data						Spectrophotometrically Determined Eleostearic Acids			
	Free Fatty Acids	$N_D^{25°C.}$	Iodine No. Wijs	Brown Heat Test	Maleic Anhydride Value	Eleostearic Acid	*Alpha*	*Beta*	Total: *Alpha* plus *Beta*	Total: Determined
	%			*Minutes*		%	%	%	%	%
1. Irradiated	......	......	......	......	69.3	77.4	12.1	60.8	72.9	71.7
2. Filtered and stored	......	......	......	......	70.5	78.8	74.8	5.1	79.9	79.6
3. From stored nuts	0.80	1.5192	164.9	9.25	71.6	80.6	79.9	2.9	82.8	83.5
4. Freshly pressed	0.28	1.5189	164.8	9.50	71.4	79.8	80.8	0.7	81.5	82.1
5. Solvent extracted	0.65	1.5182	161.7	......	68.4	76.4	34.8	39.1	73.9	75.2
6. Solvent extracted	1.00	1.5172	163.6	10.50	68.6	76.6	59.8	17.8	77.6	77.7
7. Solvent extracted	0.48	1.5172	163.8	10.75	68.4	76.4	59.4	17.7	77.1	77.1
8. Solvent extracted	0.31	1.5175	161.4	10.50	68.7	76.8	74.0	3.7	77.7	77.5
9. Solvent extracted	0.61	1.5176	161.5	10.50	67.8	75.8	36.4	37.1	73.5	74.3

form is substantially greater than that of the *alpha* form. Comparisons of the results for the total content, computed from independent measurements at 276.5 mμ, with those obtained by adding the two individually determined isomers (Table 2) show that they agree generally to within about one per cent. The discrepancies are due to a combination of the errors involved in both the simultaneous solution for the individual isomers and in the measurements at the point of intersection for the independent determination. Mathematically it can be shown that the ideal conditions for spectrophotometric binary analysis are obtained when the differences between the magnitudes of the extinction coefficients at a given wave length are at a maximum (7). Study of Fig. 1, A and B, shows that such ideal conditions are not possible in the determination of the two isomers of eleostearic acid in tung oil. Error due to the critical dependency of wave-length setting and to spectrum band isolation has already been pointed out.

The values for total eleostearic acid in various tung oils obtained spectrophotometrically are compared in Table 2 with those obtained by chemical means based on maleic anhydride values (14). The two sets of values are only in fair agreement. In some cases the differences may be due to the impossibility of making the two evaluations at the same time. Studies described later indicate that small samples of these oils may undergo some change in ratio between the *alpha* and *beta* isomers upon storage.

Repeated determinations of *alpha* and *beta* eleostearic acids in a tung oil sample illustrate the precision with which the spectrophotometric measurements enable the percentages of these acids to be calculated. The data in Table 3 show that a reproducibility of within about one per cent is attainable.

TABLE 3

Tests of the Precision of the Spectrophotometric Method for the Determination of *Alpha*, *Beta*, and Total Eleostearic Acids

Eleostearic Acid	Replicate 1	2	3	4	5	6	Average
	%	%	%	%	%	%	%
Sample No. 1							
Alpha	47.4	47.1	47.6	48.0	47.0	47.0	47.4
Beta	25.1	24.8	24.5	24.0	25.5	25.5	24.9
Total	72.5	71.9	72.1	72.0	72.5	72.5	72.3
Total[1]	72.8	72.2	72.3	71.9	72.9	72.6	72.4
Sample No. 2							
Alpha	50.3	50.2	49.2	49.6	49.1	49.8	49.7
Beta	24.8	25.2	25.5	25.7	26.5	25.9	25.6
Total	75.1	75.4	74.7	75.3	75.6	75.7	75.3
Total[1]	75.5	75.2	75.2	75.8	74.6	74.6	75.2

[1] By determination.

Several of the samples analyzed were stored for a period of from two to four months in half-filled small vials under laboratory conditions and the analyses repeated to permit some measure of changes which may occur when small samples are stored. The results are given in Table 4. They show that there is a tendency for the amount of the *alpha* isomer to decrease and of the *beta* to increase, probably by slow isomerization of the former to the latter. There is also a definite decrease in the total eleostearic acid content, probably due to polymerization. Entirely different results might be obtained if stored carload lots of the oil were repeatedly sampled and analyzed.

TABLE 4

Eleostearic Acids in Stored Samples of Tung Oil

Tung Oil Sample	Before Storage		After Storage	
	Alpha	*Beta*	*Alpha*	*Beta*
	%	%	%	%
1. Irradiated	12.1	60.8	11.4	61.5
2. Filtered	74.8	5.1	70.1	9.5
3.	79.9	2.9	76.5	3.2
4.	80.8	0.7	75.8	3.0
5. Solvent extracted	34.8	39.1	20.7	48.6
6. Solvent extracted	59.8	17.8	39.3	32.6
7. Solvent extracted	59.4	17.7	41.1	29.8
8. Solvent extracted	74.0	3.7	59.8	16.0
9. Solvent extracted	36.4	37.1	22.5	44.1

The method described offers a reasonably accurate and rapid means of determining *alpha*, *beta*, and total eleostearic acids in all types of tung oils.

REFERENCES

1. Hilditch, T. P. Chemical Constitution of Natural Fats, pp. 132-3, 221-2. New York. John Wiley and Sons. (1941).
2. O'Connor, R. T., Heinzelman, D. C., Freeman, A. F., and Pack, F. C. Ind. Eng. Chem., Anal. Ed., *17*, 467 (1945).
3. Dingwall, A., and Thomson, J. C. J. Am. Chem. Soc., *56*, 899 (1934).
4. Brode, W. R., Patterson, J. W., Brown, J. B., and Frankel, J. Ind. Eng. Chem., Anal. Ed., *16*, 77 (1944).
5. Twyman, F., and Allsop, C. B. The Practice of Absorption Spectrophotometry With Hilger Instruments, p. 41, 2nd Ed., London. Adam Hilger Ltd. (1934).
6. Miller, E. S. Quantitative Biological Spectroscopy, p. 167, Minneapolis, Minn. Burgess Publishing Co. (1939).
7. Loofbourow, J. R. Physical Methods for the Identification and Assay of Vitamins + Hormones. Vitamins + Hormones. Vol. 1. Ed. by Harris, R. S., and Thimann, K. V. New York. Academic Press, Inc., Publishers. (1943).
8. Nicolet, B. H. J. Am. Chem. Soc., *43*, 938 (1921).
9. Morrell, R. S. J. Chem. Soc., *101*, 2082 (1912).
10. Graff, M. M., O'Connor, R. T., and Skau, E. L. Ind. Eng. Chem., Anal. Ed., *16*, 556 (1944).
11. Hulst, L. J. N. van der. Rec. trav. chim., *54*, 639 (1935).
12. Miller, E. S., and Kass, J. P. Presented at A. C. S. Meeting, St. Louis, Mo. April, 1941.
13. Kass, J. P. Protective and Decorative Coatings. Vol. IV. Chapter 12. Ed. by J. J. Matiello. New York. John Wiley and Sons. (1944).
14. McKinney, R. S., Halbrook, N. J., and Rose, W. Gordon. Oil & Soap, *19*, 141 (1942).