Spectrophotometric and Chromatographic Investigations of the Oil of Sapium Sebiferum*†

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THE oil obtained from the fruits of the Chinese tallow tree, *Sapium Sebiferum,* is commonly known as stillingia oil although this name is incorrect botanically. This oil, formerly used as an illuminating oil in the Orient, offers possibility of development as a drying oil because of its high unsaturation and its drying qualities (1). A fractionation of this oil into more or less unsaturated components may yield products more suitable as drying and edible oils respectively. Chromatographic fractionation similar to that of Reinbold and Dutton on soybean oil and its esters (2) was thought promising enough to attempt.

Unpublished spectral data on the oil (3) and the unusually high negative optical rotation suggest the presence of some unknown constituent (or constituents) in the oil. A further study of the spectral absorption and an attempt to fractionate the ethyl esters of the stillingia acids seemed likely to yield some information regarding the unknown constituent.

Experimental

The oil used in this investigation was extracted from seed gathered in Houston, Texas, The seed coat and the adhering tallow were removed by stirring the seed rapidly in water at 60°C. The oil was then extracted from the ground seeds by petroleum ether. Preliminary spectral analyses of oil prepared in this way showed no difference between this product and an oil obtained by extraction of the meats separated from the shell. The bulk sample of oil used in these studies had a Wijs iodine number of 177.7 and a peroxide value of 1.4 m. eq./kg. No evidence of oxidation was noted in the absorption spectrum.

Absorption spectra of the oil in petroleum ether solution were measured, using the Beckman Spectrophotometer. Analysis of the oil for unsaturated fatty acids by alkaline conjugation was made by the method of Brice and Swain (4).

The chromatographic separation was carried out in a manner similar to that used by Dutton and Reinbold in their study with soybean oil (2) However, the chromatographic column consisted of three coupled filters, the effect of which is to sharpen the fronts in the chromatogram (5). These have been successfully used on a much smaller scale in the separation of fatty acids (6). The column was packed with 420 gm. of Harshaw Al-2 alumina and celite analytical Filter Aid mixed in a ratio of 10 to 1. Filters were packed wet and were washed with 2 liters petroleum ether before 25 gm. of stillingia oil in 100 ml. petroleum ether was forced into the column. The eluant, consisting of 15% ethyl ether in petroleum ether, was then forced through the column under pressure of CO_2. Total time of operation was 13.5 hours. The change in composition of the eluate was followed during the course of the experiment by observation of the change in refractive index of the eluate by means of a dipping refractometer placed in a specially designed cuvette allowing constant observation of the flowing liquid. The apparatus used is shown in Fig. 1. In the chromatographic separation of the ethyl esters of stillingia acids, alcohol was used as solvent, and the esters were displaced by a picric acid solution.

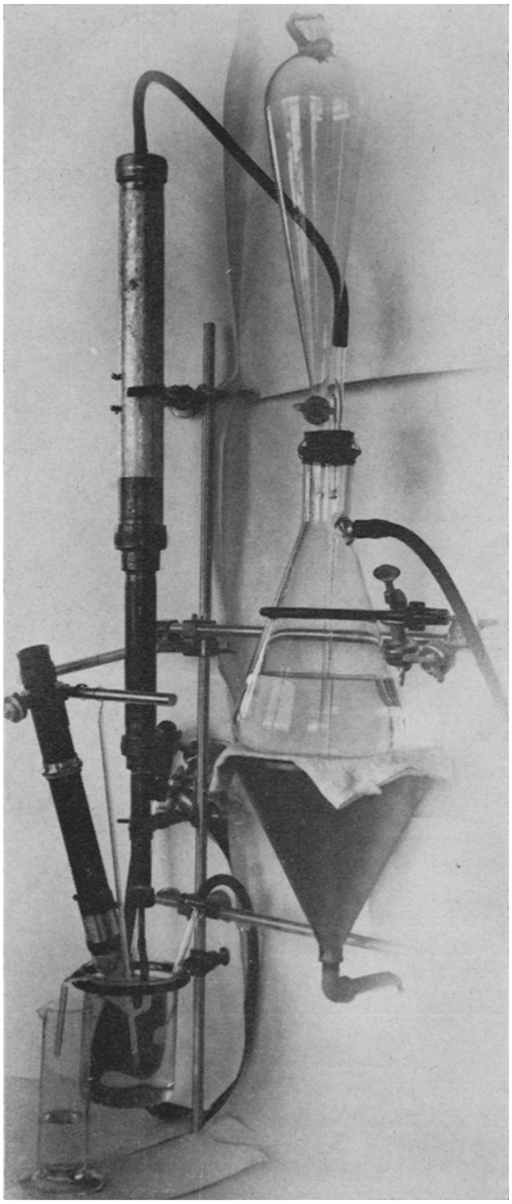

FIG. 1. Apparatus used for chromatographic separation of glycerides and esters.

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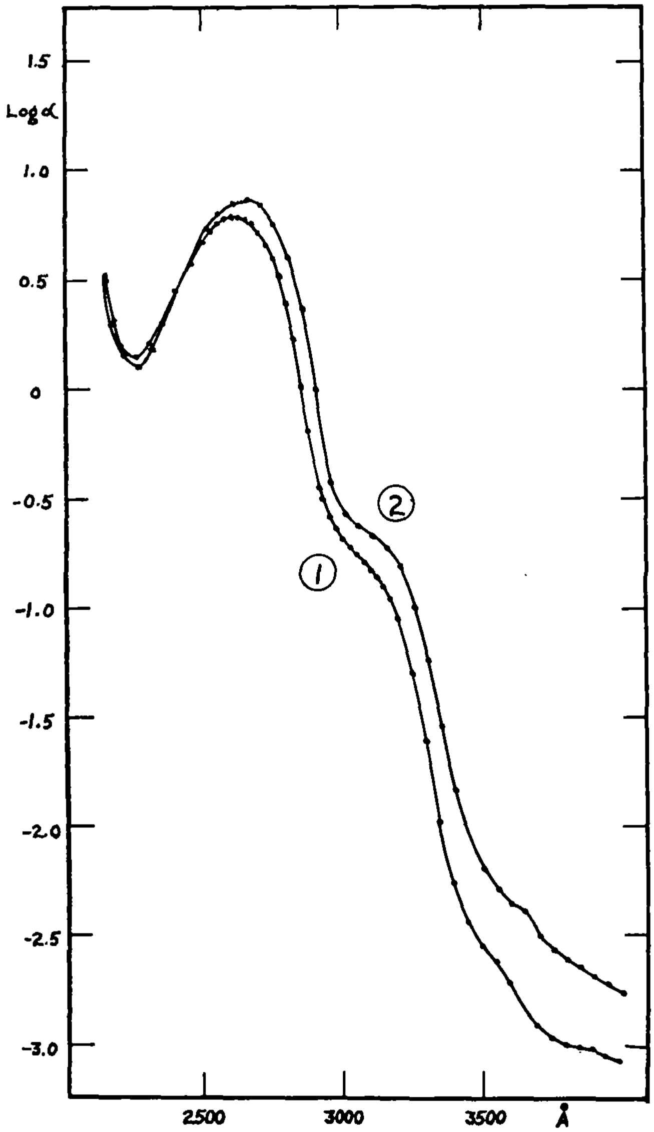

FIG. 2. Absorption spectra of stillingia oil and acids.
1. Stillingia oil in petroleum ether solution.
2. Stillingia acids in alcohol solution.

Results and Conclusions

Absorption Spectra. The absorption spectra of stillingia oil, shown in Fig. 2, has a shape unusual for fats or oils. The oil has a smooth absorption band with a maximum at 2600 Å and an inflection at 3100 Å. The low absorption at 2300 Å indicates the absence of conjugated dienes. The unique absorption band is associated with a fatty acid constituent because the fatty acids prepared from the oil showed the same band. A typical sample of acids had an absorption at 2600 Å of $\alpha = 7.1$. The smooth nature of the absorption band at first suggested the possibility of the presence of a ketonic acid. As yet attempts to prepare a 2,4-dinitro-phenylhydrazone have been unsuccessful, and treatment of the acids with alkali produces no enolization shift in the absorption band.

Alkaline isomerization of the oil leads to the development of strong absorption bands characteristic of diene and triene conjugation. However, the presence of the unique absorption band in the oil itself complicates the calculation of linoleic and linolenic acid contents. Calculations using Beadle's simpler method (7) indicate the presence of 44.9% linolenic, 26.0% linoleic, 1.8% oleic, and 27.3% saturated acids assuming the destruction of the chromophore present in the fresh oil during the high temperature alkaline treatment. On the other hand, if it is assumed this constituent is stable during the isomerization, the composition is 32.2% linolenic, 32.2% linoleic, 4.4% oleic, and 31.2% saturated acids. Until the unknown constituent is isolated and its behavior studied under alkaline isomerization conditions, the analysis by alkaline isomerization cannot be applied to stillingia oil.

Optical Rotation. The sample of stillingia oil available showed a specific rotation of —5° 1′ in chloroform solution. The asymmetric center could be present in the unsaponifiable fraction, in a component fatty acid, or in the glycerin portion of an unsymmetrical triglyceride. Saponification destroyed the optical activity, neither fatty acids nor unsaponifiable fraction possessing any rotatory property. This destruction could be the result of hydrolysis of an unsymmetrical glyceride or of racemization as the result of alkaline treatment. In an effort to decide which is the case a sample of ethyl esters was pre-

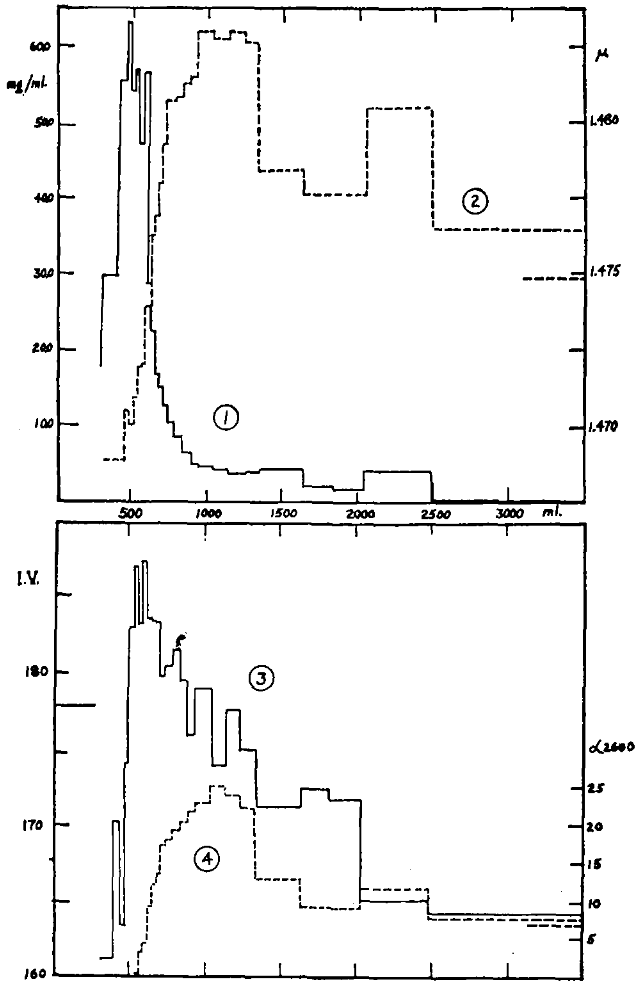

FIG. 3. Chromatographic separation of stillingia glycerides.
1. Concentration of oil in eluate, mg./ml.
2. Refractive indices of oil fractions (Abbe).
3. Iodine value of oil fractions.
4. Light absorption of oil fractions at 2600 Å.

pared by acid alcoholysis and showed a specific rotation of —2° 18′, indicating the probability that the asymmetric center is in a fatty acid. The possibility still remains that sterols racemized by alkali but soluble in the esters could be responsible for the rotation.

Chromatographic Separation of Stillingia Glycerides. The chromatographic fractionation of stillingia oil in petroleum ether on alumina indicates the possibility of separating the oil into fractions somewhat higher and lower in unsaturation than the original oil. The differential leaves much to be desired, however. A comparison of the concentration distribution, the refractive indices of the fractions as determined with an Abbe refractometer, iodine value of fractions, and the light absorption of the fractions at 2600 Å indicates that the glycerides were separated into quite different components. The initial large fraction eluted was highly unsaturated but had relatively lower refractive index and light absorption than later fractions. Optical rotations could not be determined on the fractions because of their small size. In another chromatographic separation experiment the pooled fractions corresponding roughly to the first 1000 ml. eluate in this experiment showed a specific rotation of —2° 50′ whereas the pooled samples for the remainder of the experiment had a specific rotation of —6° 6′. Thus the optical rotation seems to be associated with the high refractive index. Measurement of the light absorption of the fractions at 2600 Å indicated that the glycerides bearing the chromophoric groups are eluted somewhat later than the highly unsaturated fraction. The spectral absorption seems also to be associated with the high refractive index. At an eluate volume of 1850 ml. the eluent was changed to ether which removed more strongly adsorbed glycerides from the column. The total recovery of oil was 83%.

Chromatographic Separation of Ethyl Esters. An attempt to separate a chromophore-bearing ester was made, using the displacement technique with absolute alcohol as solvent and picric acid as displacer. The refractive index of the eluate as measured by the dipping refractometer and the light absorption at 2600 Å of the eluate fractions are plotted together in Fig. 4. It will be seen that the esters can be separated into a large initial fraction carrying the light absorbing group and into numerous later fractions in which the light absorption is negligible. It would thus appear that this chromatographic separation, which was in effect an elution rather than a displacement, was quite successful in concentrating the chromophoric constituent.

Properties of the Unknown Constituent. The substance responsible for the unusual ultraviolet absorption spectrum in stillingia oil is found in the fatty acid fraction. The chromophore is not influenced by alkali and a concentrate of the substance did not yield a 2,4-dinitro-phenylhydrazone. It thus appears that the chromophore is not ketonic. The maximum at 2600 Å and the inflection at 3100 Å appear to be due to one substance because the ratios between these two absorptions remained the same in the chromatographic fractions of stillingia oil.

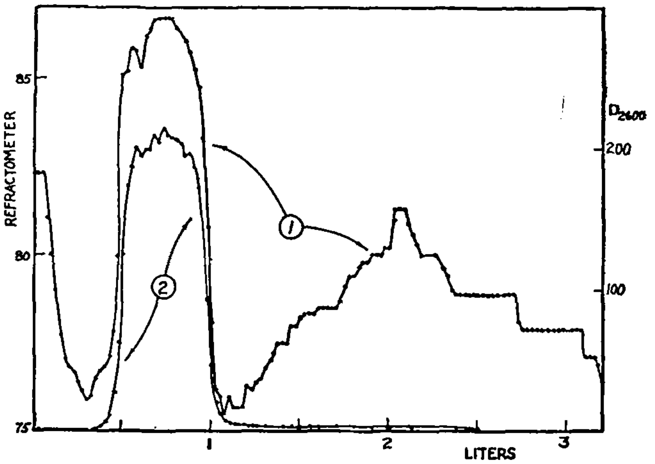

FIG. 4. Chromatographic separation of stillingia ethyl esters.
1. Refractive index of eluate by dipping refractometer.
2. Light absorption of fractions at 2600 Å.

The chromatographic studies on stillingia oil suggest that the chromophore bearing acid is associated with optical activity and high refractive index. Exposure of stillingia oil to 180° in vacuum-sealed ampules resulted in the reduction of optical activity and light absorption at 2600 Å. The optical activity decreased 80% in 10 hours whereas the light absorption decreased only 25%. The absorption spectrum of the sample heated 10 hours showed a general flattening of the curve from 2200-2600 Å, absorption due to the native chromophore being decreased, and absorption at the lower wavelength being increased. A simultaneous increase in the region above 3200 Å was also observed.

Since this manuscript was prepared, Hilditch (J. Oil Colour Chem. Assn. *32,* 5, 1949) has reported studies on stillingia oil in which the spectrum has been determined. He also established the structure of the light absorbing short chain acid as deca-2,4-dienoic acid. Subsequent studies from our laboratory indicate the multiple nature of the chromophoric substance.

Summary

1. Absorption spectra of stillingia oil and its acids indicate the presence of a new fatty acid with a smooth absorption band having a maximum at 2600 Å and an inflection at 3100 Å. This light absorptive property appears to be associated with the optical activity and high refractive index. The light absorption and optical activity are reduced by heat in the absence of air.

2. The unknown constituent can be concentrated by chromatographic adsorption of the ethyl esters in alcohol solution on an alumina column.

3. Chromatography of stillingia oil indicates that it can be separated into fractions having widely different properties.

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