# Characterization of Cyclopropenoid Acids in Selected Seed Oils

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Evidence is provided that sterculic and malvalic acids occur together in seed oils of Sterculia foetida, Hibiscus syriacus, and Lavatera trimestris. Sterculia foetida oil contains 54.5% sterculic and 6.7% malvalic acids; Hibiscus syriacus oil contains 16.3% malvalic and 3.4% sterculic; and Lavatera trimestris oil contains 7.7% malvalie and 0.6% sterculic acids. Hibiscus syriacus oil also contains 1.5% dihydrosterculic acid. The cyclopropenoid acids were characterized by hydrogenation in conjunction with gas-liquid chromatography and by oxidation to  $\beta$ -dioxo acids with subsequent cleavage with peracetic acid. Acetolysis of epoxides in the presence of cyclopropenes was effected by room temperature treatment with acetic acid-10% sulfuric acid (5:2).

**T** WO CYCLOPROPENOID ACIDS are known in nature sterculic acid (Ia), the chief fatty acid of Sterculia foetida oil isolated and characterized by Nunn (1), and malvalic acid (Ib), found in Malva seed oils (2,3,4). Seed oils of the Malvaceae other than those of Malva are known to contain acids similar to sterculic acid. These oils give a positive Halphen test (5), show infrared absorption maxima at 9.92  $\mu$  (6), and absorb hydrogen bromide when titrated by the Durbetaki method (7).

The present investigation, concerning which a preliminary communication appeared earlier (8), establishes malvalic acid as the chief cyclopropenoid fatty acid in two malvaceous seed oils-Hibiscus syriacus and Lavatera trimestris. Each oil also contains a smaller amount of sterculic acid. Our work also establishes that sterculic acid is accompanied by a smaller amount of malvalic acid in Sterculia foetida oil. Shenstone and Vickery (9) have obtained com-parable results with *Sterculia foetida* seed oil; they have also shown that malvalic and sterculic acids occur together in leaf oils of two Malva species and in cottonseed oil (both family Malvaceae) as well as in seed oils of two Brachychiton species (Sterculiaceae). They found that malvalic acid is the predominant cyclopropene in all of these except Sterculia oil (4,9).

Evidence for a cyclopropane-containing acid in  $Hibiscus \ syriacus$  oil was also obtained. This is presumably dihydrosterculic acid. An isomeric cyclopropanoid acid—lactobacillic acid—was discovered in Lactobacillus lipids by Hofmann and co-workers (10,11). Although the evidence is less definite,  $H. \ syriacus$  oil apparently also contains dihydromalvalic acid.

The occurrence of sterculic and malvalic acids in the same oils together with their dihydro derivatives presents a biogenetic problem of considerable interest. Fatty acids in naturally occurring glycerides, plant or animal, are almost always found in homologous series with even-numbered chains differing in length by multiples of two carbons. In contrast, sterculic and malvalic acids differ in chain length by a single carbon; malvalic acid has an odd-numbered

<sup>1</sup>This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.  $(C_{17})$  chain. We suggest the following as a possible scheme for biosynthesis of cyclopropenoid acids:

$$\begin{array}{ccc} -\mathrm{CH}=\mathrm{CH}- & + & :\mathrm{CH}_2 & \longrightarrow & -\mathrm{CH}-\mathrm{CH}- & \frac{-2\mathrm{H}}{\overset{}{\underset{\mathrm{CH}_2}} & -\mathrm{C}=\mathrm{C}-\\ & & & & & \\ \mathbf{A} & & & \mathbf{B} & & \mathbf{C} \end{array}$$

Microbial synthesis of lactobacillic acid by addition of a one-carbon fragment across the double bond of *cis*-11-octadecenoic (*cis*-vaccenic) acid  $(A \rightarrow B)$  has been demonstrated by Hofmann and Liu (12). A similar conversion of oleic to dihydrosterculic acid has been reported by Bloch and co-workers (13). Desaturation of stearic to oleic acid, analogous to conversion  $(B \rightarrow C)$ , has also been demonstrated (14,15). Thus oleic acid would serve as the biosynthetic precursor of sterculic acid, with dihydrosterculic acid as an intermediate. Such a scheme would require a  $C_{17}$ -monoethenoic acid as a precursor for malvalic acid.  $C_{17}$ -acids in lipids of higher plants are rare but it was noted that oils containing malvalic acid also contain in some cases a measurable amount of a heptadecenoic acid (see Tables I and II).

Another biogenetic oddity associated with malvaceous seed oils is that they frequently contain epoxy acids as well as cyclopropenes (16). Hopkins and Chisholm characterized the epoxy acid in *Hibiscus* and *Lavatera* oils as the enantiomer of epoxyoleic acid that yields (+)-threo-12,13-dihydroxyoleic acid when treated with acetic acid.

Cyclopropenoid acids in seed oils were determined by hydrogen bromide titration and differentiated from epoxy acids by lithium aluminum hydride reduction (17). Gas liquid chromatographic (GLC) analysis showed that malvalic acid peaks were masked by linoleic acid peaks, but hydrogenation studies were used to estimate the quantity of malvalic and sterculic acids in the original oil. The values obtained by hydrogenation are in fair agreement with the values obtained by titration with hydrogen bromide (Table III).

Prolonged hydrogenation of cyclopropene-containing acids (or oils) in ethanol produced dihydro derivatives and branched-chain hydrogenolysis products, but none having straight chains. In acetic acid, however, both branched-chain and straight-chain hydrogenolysis products were produced. The dihydro derivatives were probably cis-DL-9,10-methyleneoctadecanoic (18), and cis-DL-8,9-methyleneheptadecanoic acids (3). Lactobacillic acid was shown to be cis-D or L-11,12-methyleneoctadecanoic acid by Craven and Jeffrey (19). The branched-chain hydrogenolysis products were probably 8-(9-)methylheptadecanoic and 9-(10-)methyloctadecanoic acids according to the results obtained by Hofmann and co-workers (10). The straight-chain hydrogenolysis product from sterculic acid was nonadecanoic acid.

Our attempts at isolating malvalic acid from *Hibis*cus oil by low-temperature crystallization or by urea

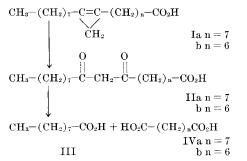
TABLE I
GLC Analysis of Fatty Acids (Methyl Esters) from Seed Oils Containing Cyclopropenoid Acids

Acid	Sterculia foetida	Hibiscus syriacus	Lavatera trimestris
	%	%	%c
Lauric			0.2
Myristic	0.1	0.2	0.3
Palmitic	20.5	17.0	18.1
Palmitoleic	0.3	0.8	0.4
C17 mongene		0.5	0.7
Stearic	3.2	1.6	3.1
Oleic	11.0	13.0	27.0
Linoleic	12.2	56.5	44.1
Linolenic	0.5	0.5	0.2
Sterculic	50.0	5.2	0.9
Dihydrosterculic		1.5	
Epoxyoleic		1.3	3.2
Unknown.	2.3	1.2	1.6

TABLE II GLC Analysis of Hydrogenated Fatty Acids (Methyl Esters) from Seed Oils Containing Cyclopropenoid Acids

Acid	Hibiscus syriacus	Lavatera trimestris		Sterculia foetida oilª
	%	%	%	%
Myristic	0.4	0.2	0.1	
Palmitic	17.1	17.5	17.1	18.8
Heptadecanoic	1.8	1.2	0.6	
8. (9.) Methylheptadecanoic	5.8	2.5	1.0	1.2
Dihydromalvalic	10.1	5.2	5.7	4.6
Malvalic	0.4			
Stearic	59.7	67.6	20.6	13.1
Dihydrosterculic	3.5	0.6	45.7	38.5
9-(10-) Methylstearic	1.4		8.8	11.5
Hydroxystearic		2.8		
Nonadecanoic				11.5
Ünknown		2.2		

adduction were unsuccessful. The incomplete separation of cyclopropenoid acids from linoleic by these techniques may be attributed to large quantities of linoleic acid present in malvaceous seed oils. Because of the difficulties encountered in isolation techniques, the cyclopropenoid acids in *Hibiscus* and *Lavatera* were characterized indirectly according to the following scheme:



Van Rudloff's periodate-permanganate oxidation procedures (20,21) were applied to cyclopropenoid acids and oils. The expected cleavage  $(I \rightarrow II)$  occurred, but it was often complicated by overoxidation  $(II \rightarrow III + IV)$  or by formation of small amounts of other by-products. In some cases, a small amount of product was formed having an infrared maximum at 5.6  $\mu$ , suggesting a four-membered ring ketone. Such a compound could result from an intramolecular aldol condensation of a  $\beta$ -diketone.

Periodate-permanganate oxidized (21) *H. syriacus* oil after saponification yielded a mixture containing 8,10-dioxoöctadecanoic, 9,11-dioxononadecanoic, dihydrosterculic, and dihydromalvalic acids.

The material isolated by chelation contained 87.8% C<sub>18</sub> dioxoester and 12.2% C<sub>19</sub> dioxoester. The mixture of dioxoesters upon saponification followed by per-

TABLE III Percentages of Hydrogen Bromide-Reactive Acids

	GLC analysis			
Species	Stercu- lic acid <sup>a</sup>	Malva- lic <sup>b</sup> acid	Epoxy acid	HBE ¢
	%	%	%	
S. foetida H. syriacus L. trimestris	54.5 3.4ª 0.6		$1.3 \\ 3.2$	$57.6 \\ 21.9 \\ 11.9$

<sup>a</sup> Value has been corrected for dihydrosterculic acid found in the original oil. <sup>b</sup> Values were obtained indirectly from products formed by hydrogenation of the fatty acids. <sup>c</sup> Hydrogen bromide equivalent (HBE) is a measure of hydrogen bromide consumption expressed as moles consumed per mole of Cis-acid (eq. wt. 296.5) times 100.

acetic acid oxidation (1) yielded pelargonic 28.0% (45.5% of theory), octanedioic 52.0% (87.0% of theory), nonanedioic 12.0% (100% of theory), and 12 minor components 8%.

We found that cyclopropenes are largely destroyed by Gunstone's procedure (22) for acetolysis of fatty acid epoxides (treatment with boiling acetic acid). The nature of acetolysis products derived from sterculic acid has been elucidated by Rinehart and coworkers (23). Acetolysis of epoxides of *Lavatera trimestris* seed oil was effected without destruction of the cyclopropene moiety by treatment with acetic acid-10% sulfuric acid (5:2) at room temperature. Saponification followed by removal of the dihydroxy acids yielded acids with a hydrogen bromide equivalent (HBE) of 8.5. Hydrogenation studies and GLC analysis showed that this fraction contained 8.3% cyclopropenoid acids (Table II).

Ozonolysis of the acids (HBE 8.5),  $(I \rightarrow II)$ , yielded a concentrate showing  $\lambda_{max}$  275 m $\mu$  (E 50.0) after removal of aldehydes and aldehydo acids. The dioxoesters (II) were isolated by chelation with cupric acetate and oxidized with peracetic acid (II  $\rightarrow$ III + IV). GLC analysis of the oxidation products indicated the major acids were pelargonic, octanedioic, and nonanedioic. A small quantity of heptanedioic acid was also observed, which probably was produced as a degradation product of octanedioic acid.

#### Experimental

Apparatus and Methods. GLC analyses were carried out using a Burrell Kromo-Tog K-5 instrument as described by Miwa and co-workers (24). Equivalent chain length (ECL) values used in identifying methyl esters of acids not reported by Miwa and co-workers are found in Table IV. The methyl esters were prepared by the diazomethane method. Hydrogenations were carried out in ethanol using 10% palladium on carbon unless otherwise specified. Ultraviolet absorption measurements were made in absolute ethanol using a Beckman model DU spectrophotometer.

Extraction and Saponification of Oils. Coarsely ground seeds of Hibiscus syriacus and Lavatera trimestris were extracted overnight with petroleum ether (b.p. 30-60°C.) using Soxhlet extractors. Removal of solvent in the usual way yielded 27.0% oil (HBE 21.9) from H. syriacus and 15.5% oil (HBE 11.9) from L. trimestris. Sterculia foetida oil (HBE 57.6) was obtained in 51% yield by petroleum ether extraction of crushed nuts at room temperature.

Saponification was effected by stirring the oils with 0.8 N alcoholic KOH overnight at room temperature. The unsaponifiable material was removed. The com-

 TABLE IV

 Equivalent Chain Lengths (ECL) of Methyl Esters

Acid	ECL		
Aciu	Apiezon-L	Resoflex-446	
8-(9-) Methylheptadecanoic	17.3	17.2	
Dihydromalvalic	17.8	18.4	
9-(10-) Methyloctadecanoic	18.3	18.2	
Dihydrosterculic	18.8	19.4	
8,10-Dioxoöctadecanoic	20.6		
9,11-Dioxononadecanoic	21.6		

position of the mixed acids obtained from various oils is shown in Table I.

Estimation of Percentages of Acids by Hydrogenation. In a typical preparation, a 0.100-g. portion of fatty acids was hydrogenated at 1 atm. for 30 to 45 min. using 0.010 g. of catalyst at room temperature. The catalyst was removed and the solvent was evaporated, yielding solid or semisolid acids. Iodine value determinations showed whether the hydrogenations were complete. The composition of the hydrogenated acids as methyl esters is shown in Table II.

Hydrogenation of cyclopropene-containing acids (or oils) using Adams' catalyst in ethanol did not produce straight-chain hydrogenolysis products. However, branched-chain hydrogenolysis products were produced. Nonadecanoic acid and also branched-chain hydrogenolysis products were produced by hydrogenation of a sample of *Sterculia foetida* oil for 3 hr. using Adams' catalyst in glacial acetic acid followed by saponification in the usual manner. Table II shows the results obtained from prolonged hydrogenations.

Characterization of the Acids in Hibiscus syriacus Seed Oil. Attempts to isolate the acids by urea adduction according to the procedure of Nunn (1) resulted in mixtures of malvalic contaminated with linoleic acid. A combination of low-temperature crystallization and urea adduction produced a mixture with HBE no higher than 57.0.

A 5.00-g. portion of H. syriacus oil was stirred overnight with 34.80 g. of sodium periodate, 2.10 g. of potassium carbonate, and 0.653 g. of potassium permanganate in 2,000 ml. of 60% t-butyl alcohol for 24 hr. The pH was 6.9 at the start of the reaction and 6.3 upon completion of the reaction. The organic layer was removed then the aqueous layer was decolorized with sodium bisulfite and extracted with ether. The organic layer and the ether extracts were combined, dried over sodium sulfate, and evaporated in vacuo; the mixture yielded 3.02 g. of oil with an iodine value of 10.5. The iodine value of the original oil was 121. This oil (3.02 g.) was saponified at room temperature by treatment overnight with 0.8 N alcoholic potassium hydroxide; 2.52 g. of fatty acids were isolated by acidification and ether extraction. These semisolid fatty acids (2.52 g.) were triturated with (10 x 25 cm)ml.) portions of petroleum ether; evaporation of the p.e. extracts yielded 1.24 g. of a semisolid giving a dark red color with 3% alcoholic ferric chloride. GLC analysis showed that the mixture contained 9.9%  $C_{18}$ dioxo, 1.1% C<sub>19</sub> dioxo, 1.7% dihydrosterculic, and 3.9% dihydromalvalic acids. A 0.399-g. portion of this esterified mixture was treated with 5 ml. of a saturated solution of cupric acetate in methanol essentially according to the procedure of Faure and Smith (6). A blue-gray precipitate was formed. The crystals were collected on filter paper, washed with cold methanol, and dried by suction-yield, 0.095 g., m.p. 92-93°C. The dioxoesters were liberated from the copper chelate by acidification with concentrated hydrochloric acid and ether extraction. The yield of dioxoesters was 0.078 g. The ultraviolet spectrum of this material showed maximum absorption at 275 mµ ( $\epsilon$  9,100; calcd. as C<sub>19</sub> diketo acid). Nunn (1) reported  $\epsilon$  10,500 at 275 mµ for 9,11-dioxononadecanoic acid. The infrared spectrum of the dioxoesters in carbon disulfide showed a peak at 6.25 µ ( $\beta$ -diketone). According to GLC analysis the sample was 87.8% dioxoöctadecanoate and 12.2% dioxononadecanoate. This mixture of esters was saponified then oxidized with peracetic acid (1). The oxidation mixture contained pelargonic 28.0% (45.5% of theory), octanedioic 52.0% (87.0% of theory), and nonanedioic acid 12.0% (100% of theory), plus 8% of 12 minor components according to GLC analysis.

Acetolysis of Epoxides. Gunstone's procedure (22) for the acetolysis of epoxides (boiling acetic acid) was applied to L. trimestris oil but resulted in destruction of the cyclopropene ring according to titration with hydrogen bromide. Preliminary experiments with H. syriacus and Vernonia anthelmintica seed oils showed that acetolysis could be effected with acetic acid-10% sulfuric (5:2) without appreciable loss of the cyclopropenoid moiety. A 0.514-g. portion of V. anthelmintica oil (HBE 71.6) was stirred overnight at room temperature with 2 ml. of 10% sulfuric acid in 5 ml. of acetic acid. The solution was diluted with water and extracted with ether. The ether extracts were washed with water, dried over sodium sulfate, and evaporated to yield a viscous oil (HBE 0.01). A 0.546-g. portion of H. syriacus oil (HBE 22.0) was treated similarly with acetic acid-10% sulfuric acid. The product was worked up in the same manner and yielded an oil, HBE 21.9.

Isolation of the Acids in Lavatera trimestris Seed Oil. Oil recovered from the acetic acid-sulfuric acid treatment was saponified in the usual way. Acids liberated from the mixture were partitioned between petroleum ether and 80% methanol as described by Gunstone (22). The bulk of the acids were obtained free of dihydroxy acids in the residue obtained by evaporating the combined petroleum ether layers (HBE 8.5). A portion of these acids was hydrogenated and was shown to contain 5.2% dihydrosterculie, 2.5% 8-(9-)methylheptadecanoic and 0.6% dihydrosterculic, by GLC analysis. This 8.3% of sterculictype acids in the petroleum ether fraction is in close agreement with the value obtained by hydrogen bromide titration.

Characterization of Acids in L. trimestris Seed Oil. A 5.03-g. portion of malvalic acid from the petroleum ether layers (HBE 8.5) was ozonized in 150 ml. ethyl acetate at  $-25^{\circ}$ C. for 13.5 min. according to the procedure of Nunn (1). The ozonide was decomposed by catalytic hydrogenation, the catalyst was removed by filtration and the solution was treated with diazomethane. Removal of solvent in vacuo yielded 5.30 g. of oil. The product was dissolved in 300 ml. of ether and washed with  $(3 \times 100 \text{ ml.})$  portions of water. The ether extracts were dried over sodium sulfate and evaporated. A 2.09-g. portion of material from the ether extract was saponified with 0.8 N alcoholic KOH, and 2.36 g. of the product was isolated. The product was steam distilled for 2 hr., and the nonsteam-volatile components were isolated by ether extraction. The ether extracts were washed with (10 x 100 ml.) portions of saturated sodium bisulfite solution. Drying the ether extracts over soDECEMBER, 1961

dium sulfate and evaporating yielded 1.23 g. of a semisolid. The ultraviolet spectrum showed  $\lambda_{\max}^{EtOH}$  275  $m\mu$  (E 50.0), and the infrared spectrum showed a peak at 6.25  $\mu$  ( $\beta$ -diketone). This semisolid was esterified and treated with cupric acetate in methanol (1); the yield was 0.070 g., m.p.  $90-92^{\circ}C$ . The complex was decomposed and 0.063 g. of esters were isolated by ether extraction. Saponifying the mixture of dioxoesters yielded 0.056 g. of acids. These acids were oxidized by peracetic acid (1), and 0.037 g. of oxidation product was recovered by ether extraction. GLC analysis showed that pelargonic and octanedioic acids were the major degradation products. Nonanedioic and heptanedioic acids were also observed in smaller quantities. The heptanedioic acid may have resulted from degradation of octanedioic acid.

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### The Cuticle Wax of the Cuban Palm, Copernicia hospita

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The physical and chemical constants characterizing a previously unreported palm wax are given. These are compared to and are quite similar to those of carnauba. There is also reference to certain gross field characteristics such as leaf size, age at time of flowering, and wax yields. The wax compares favorably with carnauba in typical polish compositions.

NTENSIVE FIELD STUDIES have been underway for over 18 years to better establish the taxonomy of the West Indies and South American species of the genus Copernicia. A portion of the data gathered has recently appeared as a published monograph (1). Several of the species of this genus have been called to the authors' attention because of unusual waxy character found in the leaves.

A substantial number of plants representing various Cuban species of this genus have been grown from seed under experimental wax palm plantation conditions in northeast Brazil (2). Because it was possible to simulate typical field conditions equivalent to what the commercial Brazilian carnauba (Copernicia cerifera, Martius) wax palms receive, it has also been possible to harvest the waxes of these currently unexploited palms and draw direct comparisons to the carnauba palm. The chemical analyses for one of these palm waxes have been completed and are reported here.

The Copernicia hospita palm has been regarded from the early taxonomic studies and early field work in Cuba (3) to be an outstanding candidate for wax production. Trees of this species being grown in northeast Brazil are already showing evidence (Fig. 1) of botanical maturity (flowering) and adequate size capable of withstanding the severe pressure of annual leaf cutting harvest at the age of five years. Carnauba (C. cerifera) does not reach maturity (flowering) before 12-15 years of age and does not provide commercial wax yields before it is 8 years old.

The leaves in this study were collected using techniques commonly practiced by the native harvesters of carnauba in Brazil. They were excised from the crown and sun-dried for five days. The wax was removed from the leaves by a mechanical beating process using a Guarani-cyclone machine. In this process the dried leaves are automatically cut into pieces 2-3 in. long and beaten by revolving arms in the body of this machine and the free flaking wax powder thus dislodged is collected by a cyclone air separator. The free flaked wax was then screened using a 40-mesh sieve in order to remove most of the vegetable fragments and fibers.

A quantity of the screened powder was melted and clarified by filtration. The several routine constants generally determined on waxes were run on the clarified wax with the results tabulated below and compared with like constants on carnauba. Notable differences between the two waxes occur in the higher