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Cyclopropene Fatty Acids of Selected Seed Oils from Bombacaceae, Malvaceae, and Sterculiaceae

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ABSTRACT

Fatty acid compositions of seed oils from three species of Bombacaceae, eleven from Malvaceae, and six from Sterculiaceae were determined. Each of the seed oils contains varying amounts of both malvalic and sterculic acids accompanied by one or both of the corresponding cyclopropane fatty acids. In addition, the seed oil of *Pachira aquatic* Aubl. (Bombacaceae) contains 12.8% α-hydroxy-sterculic acid.

INTRODUCTION

Cyclopropene fatty acids have been reported as constituents in the seed oils from many species of the Malvaceae, Sterculiaceae, Tilliaceae, and Bombacaceae families (1). A number of oils from these families have been investigated and found to contain both malvalic and sterculic acids, frequently accompanied by smaller proportions of one or both of the analogous cyclopropane acids (2,3). In addition, seed oils containing malvalic acid usually contain measurable amounts of epoxy acids (4).

It is evident from these earlier investigations that seed oils containing such an array of compounds are difficult to quantitatively analyz by traditional gas liquid chromatography (GLC) and hydrogen bromide (HBr) titration procedures. Recourt et al. (5) have shown that the cyclopropene acids tend to isomerize and decompose as they pass through the GLC column. In addition, GLC data show that the malvalic acid peak is masked by the linoleic acid peak (3) and that the corresponding cyclopropane acid may also be obscured by the presence of oleic acid (6). The HBr titration methods most often used to determine the cyclopropene and epoxy acids present are recognized as being subject to interference by conjugated dienols (7).

The present investigation was undertaken to determine the fatty acid composition of a series of seed oils that contains a mixture of normal fatty acids and fatty acids with epoxy, hydroxy, cyclopropene, and cyclopropane functional groups.

EXPERIMENTAL PROCEDURES

Oil was obtained from the ground seed as previously described (8). Fatty acids in the oils reacting with hydrogen bromide at 55 C were determined by the titration method of Harris et al. (9). Methyl esters for GLC were prepared by sodium methoxide catalyzed methanolysis and treated with silver nitrate by the procedure of Schneider et al. (10) to convert the cyclopropene acids to derivatives amenable to chromatography. Epoxy acids, when present, were converted to methoxy-hydroxy derivatives by treating with boron trifluoride (11). Equivalent chain lengths (ECL) of methyl esters and the reaction products were determined by GLC as previously described (6). The normal methyl esters were separated from the reaction products of the cyclopropene and hydroxy acids by thin layer chromatography (TLC) on 1.0-mm layers of Silica Gel G developed with n-hexane-ethyl ether (70:30). The normal methyl esters were separated according to degree of unsaturation on Silica Gel G (containing 20% silver nitrate) developed with benzene. Mass spectral data were obtained with a Dupont 21-491-2 mass spectrometer interfaced to a Bendix Model 2600 gas chromatograph (GC–MS). The column, 6 ft x ¼ in. packed with 5% Silar (5 cp) was held isothermally at 195 C.

Nuclear magnetic resonance (NMR) spectra were determined with a Varian HA-100 spectrometer. Samples were dissolved in deuterochloroform; tetramethylsilane was used as an internal standard. Infrared (IR) spectra were measured on liquid films with a Perkin-Elmer Model 337 spectrometer. A Beckman DK-2A spectrophotometer was used to record ultraviolet (UV) spectra. Hydroxyl groups were silylated with bis(trimethylsilyl)-trifluoroacetamide in pyridine.

RESULTS AND DISCUSSION

Preliminary Identification of Methyl Esters and Derivatives

GLC analyses of mixed methyl esters containing only normal esters and derivatized cyclopropene esters were consistent with the peaks previously reported for the esters from derivatized *Sterculia foetida* oil (10). However, GLC of those mixed esters also containing

TABLE 1

Composition of Seed Oils

Sample	Oil in seed (%)	Fatty acid composition by GLC (area %)														HBr reactive acids at 55 C (%)	Spectral data	
										Cyclopropane		Cyclopropene					UV	IR
		16:0	16:1	17:0	17:1	18:0	18:1	18:2	20:0	20:1	18	19	18	19	Epoxy[a]	Hydroxy[b]			
Bombacaceae																			
Chorisia speciaea St. Hil.	21.7	18.7	0.5	0.2	0.2	2.8	8.4	44.7	0.7	---	0.1	0.5	12.4	10.0	0.8	---	21.0	---	---
Pachira aquatica Aubl	55.5	60.5	---	0.3	---	2.9	7.6	4.7	0.4	---	---	0.1	1.6	8.6	---	12.8	26.6	---	OH
Salmalia malabarica (DC) Schott and Endl.	23.6	30.0	0.6	0.2	0.8	5.0	16.7	25.0	---	---	tr	0.6	7.5	11.0	1.0	---	15.5	L_3[c] 1.0	---
Malvaceae																			
Althaea hirsuta L.	12.2	13.0	0.5	0.3	0.4	4.4	7.5	52.8	0.8	---	0.4	0.2	16.5	1.6	---	---	22.4	---	---
Hibiscus grandiflorus[d]	11.7	18.2	---	0.4	2.0	6.0	15.5	46.0	0.3	---	0.8	1.3	4.0	3.0	---	1.6	7.2	---	OH
Hibiscus syriacus[d]	27.7	20.0	tr	tr	0.7	2.2	10.2	42.6	0.3	---	tr	1.0	13.4	3.0	2.7	2.8	19.9	---	OH
Kitaibelia vitifolia Willd	22.6	8.0	0.3	tr	0.3	2.5	14.2	64.4	0.5	---	0.2	0.1	7.7	1.5	---	---	10.5	---	OH
Lagunaria patersonii G. Don	18.9	23.0	3.9	tr	0.4	4.7	21.5	22.3	tr	---	---	1.1	7.7	3.9	---	1.4	14.1	---	OH
avatera kashmiriana Comb.	19.1	15.0	tr	tr	0.6	3.6	10.8	50.5	0.5	0.3	0.5	tr	16.4	1.2	---	---	14.4	---	---
Malva montana Forsk	18.4	15.7	0.2	tr	0.4	3.3	10.3	55.5	---	---	tr	0.2	12.1	1.0	---	1.0	14.4	---	---
Malva parviflora L.	11.0	15.4	---	0.2	1.1	3.0	9.0	53.4	0.8	---	0.2	0.4	12.0	1.7	1.0	1.2	14.8	---	---
Malva tournefortiana L.	17.0	12.4	0.5	1.6	2.1	3.5	11.0	43.8	2.3	0.2	tr	0.3	17.8	1.5	2.0	---	23.7	---	---
Malope trifida Paxt.	17.7	18.0	0.3	0.2	1.6	3.7	6.8	44.3	0.2	---	0.2	0.3	11.5	3.1	6.5	1.2	24.9	L_2[e] 5.4	OH
Pavonia sepium St. Hil	26.6	31.0	1.0	tr	0.2	2.0	15.4	34.2	0.5	tr	tr	tr	2.3	1.3	5.1	5.7	17.6	L_2 3.6	OH
Sterculiaceae																			
Firmiana platanifolia Schott and Endl.	24.3	17.2	1.2	tr	0.2	2.4	18.2	36.0	0.7	---	tr	0.3	1.6	20.5	2.0	---	24.3	---	---
Pterygota alata Roxb.	47.8	24.4	1.6	0.2	0.3	3.3	9.9	38.5	0.6	---	0.1	0.6	12.2	2.5	2.7	1.4	19.5	---	OH
Pterospermum acerifolium (L.) Willd	21.5	17.0	tr	tr	0.3	2.9	8.6	32.3	1.0	---	---	---	32.2	3.8	---	---	39.1	---	---
Sterculia foetida L.	53.5	14.7	tr	tr	tr	1.4	4.9	4.5	1.8	0.2	tr	0.4	6.3	65.1	---	---	64.6	---	---
Tarrietia utilis[d]	29.7	29.2	0.7	0.1	0.2	2.0	20.3	18.9	0.6	tr	tr	0.2	6.8	20.2	---	---	25.7	---	---

[a]Epoxy acid is vernolic by GLC evidence.

[b]Hydroxy acid is a conjugated dienol by GLC evidence except in *P. aquatica* and *H. syriacus* which were identified as α-hydroxysterculic.

[c]L_3 conjugated triene; % in fatty acids.

[d]Authority not available.

[e]L_2 conjugated diene; % in fatty acids.

epoxy, hydroxy, or conjugated unsaturation were difficult to interpret. Esters shown to contain the epoxy function were further derivatized by converting this group to the methoxy-hydroxy derivative followed by conversion of the hydroxyl group to the trimethylsiloxy (TMS) ether (11). The resulting TMS-derivatized esters were reanalyzed by GLC. These data (Table I) indicated that the seed oils contained varying amounts of epoxy and/or hydroxy acids in addition to the cyclopropanes, cyclopropenes, and normal fatty acids. The varying amounts and wide distribution of the epoxy and hydroxy acids in these oils were as expected (1,4,12). The mixed esters derived from each oil were separated into three fractions by preparative TLC on Silica Gel G plates. GLC data indicated that fraction I was a mixture of normal long chain methyl esters and possibly the cyclopropane methyl esters. Fraction II contained the esters of the cyclopropene derivatives, and fraction III contained the hydroxy methyl esters and the methoxy-hydroxy derivatives obtained from the epoxy methyl esters. De Bruin et al. (13) reported that *Pachira aquatic* oil possibly contained an acid similar to sterculic, but which might also contain hydroxyl group(s). Therefore, the hydroxy acid component was isolated and characterized.

Characterization of the Hydroxy Acid in *P. Aquatica*

The migration pattern of the component(s) in fraction III isolated from *P. aquatica* was compared to that of a known mixture of α-hydroxy octadecanoates and methyl sterculate. The major component in the isolated material remained near the origin along with the α-hydroxy acids whereas authentic sterculate migrated nearer to the solvent front. Distinctive IR absorption bands at 1265, 1210, and 1110 cm^{-1} are indicative of α-hydroxy esters (14). Absorption bands at 1850 and 1010 cm^{-1} were also present (cyclopropene ring). A pronounced signal (singlet) in the NMR spectrum at δ4.12 suggested that a methine proton was on the carbon adjacent to the carboxyl function. Signals at δ2.3-2.4 associated with a methylene group in the alpha position were not observed. After conversion of the hydroxyl group of the major component to a TMS derivative, ECLs of 18.6 (APL column) and 20.6 (R-446 column) were observed. The ECLs were similar to those of authentic methyl sterculate (6). Mass spectral data of the isolated component were identical to those of α-hydroxy-sterculic acid of *Pachira insignis* as reported by Morris and Hall (15). The fragmentation patterns of the silver nitrate derivatives obtained from the isolated component were not as clearly defined. However, the appropriate molecular ions at m/e = 412 for the ketone derivative and m/e = 428 for the methoxy derivative were apparent. Also, the ion at m/e = 161 resulting from cleavage alpha to the carbon bearing the TMS group was observed.

Characterization of the Cyclopropene Acids

The silver nitrate derivatives obtained from the methyl esters of the cyclopropene acids were found in fraction II from TLC. The migration pattern and GLC elution pattern of these components were identical to the corresponding derivatives of malvalic and sterculic acids from the seed oil of *Sterculia foetida* by Recourt et al. (5). The MS fragmentation patterns are also identical to those reported by Eisele and co-workers (16).

Characterization of the Cyclopropane Acids

The cyclopropane acids were contained in fraction I from the preparative TLC along with the normal long chain fatty acids. These acids have retention characteristics similar to those of the analogous monounsaturated methyl esters (6). Therefore, it was necessary to further separate fraction I according to degree of unsaturation by $AgNO_3$-TLC prior to identification of the cyclopropane acids. The fraction collected as the saturated methyl esters from this mixture was shown by GLC to contain small amounts of dihydromalvalic acid with ECLs of 17.7, (APL column) and 18.4 (R-446 column) and/or dihydrosterculic acid with ECLs of 18.7 (APL column) and 19.4 (R-446 column). Mass spectra of these acids are similar to those of the parent monoenoic esters (17). The only apparent difference is that the cyclopropane acids show a molecular ion 14 mass units greater than the parent monoenoic ester.

Description of Seed Oils

Oil content of the seeds varied from 11% in *Malva parviflora* to 56% in *P. Aquatica.* The seed oils, except for three (Table I) showed no UV absorption indicative of conjugated unsaturation. Epoxy acids, if detected, ranged from 0.8% in *Chorisia speciasa* to 6.5% in *Malope trifida.* Cyclopropene acids ranged from 3.6% in *Pavonia sepium* to 71.4% in *S. foetida.* In general, the presence of a cyclopropene ring was established in the seed oils by HBr titration at 55 C and by the presence of absorption bands in the IR spectrum at 1010 and 1850 cm^{-1}. GLC analyses for the cyclopropene acids are probably more reliable than the HBr titration because of uncertainties in the titration method pointed out by Feuge et al. (18). Also

observed in the spectra was absorption at 3550 cm^{-1} (hydroxyl) from many of the oils. The seed oil of *P. aquatica* contained 12.8% α-hydroxysterculic acid.

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