A Minor Source of Vernolic, Malvalic, and Sterculic Acids in *Pithecollobium dulce* (syn. *Inga dulcis*) Seed Oil

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ABSTRACT: *Pithecollobium dulce*, Benth (syn. *Inga dulcis*, Willd) seed oil, belonging to the Leguminosae plant family, contains minor amounts of vernolic acid (12,13-epoxy-octadec-*cis*-9-enoic acid, 10.0%), malvalic acid [7-(2-octacyclopropen-1-yl)heptanoic acid, 3.2%], and sterculic acid [8-(2-octacyclopropen-1-yl)octanoic acid, 2.0%]. The other normal fatty acids are palmitic (12.1%), stearic (4.2%), behenic (10.6%), oleic (34.1%), and linoleic (23.8%). These fatty acids have been characterized by Fourier transform infrared, ^{1}H nuclear magnetic resonance, mass spectrometry and gas–liquid chromatography techniques and by chemical degradations.
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KEY WORDS: A minor source of unusual fatty acids: vernolic acid (12,13-epoxy-octadec-*cis*-9-enoic acid), malvalic acid (7-[2-octacyclopropen-1-yl]heptanoic acid) and sterculic acid (8-[2-octacyclopropen-1-yl]octanoic acid), fatty acids, Leguminosae, *Pithecollobium dulce*, Benth (syn. *Inga dulcis*, Willd), seed oil.

Seed oils containing epoxy fatty acids have been used as stabilizers for plastics formulations and in preparations of other long-chain compounds. Epoxidized vegetable oils, such as soybean and linseed oils, have some of the properties of polymeric plasticizers but with some aging properties. Many other types of plasticizers continue to be used in minor amounts because they can be applied in resins or rubbers other than polyvinyl chloride (1). New and interesting unusual fatty acids present in high concentrations of certain seed oils are being exploited for industrial utilization, especially for the production of oleochemicals (2).

Seed oils that contain cyclopropenoid fatty acids have been investigated extensively because of their biological effects on animals and their co-carcinogenic properties. The cyclopropenoid fatty acids have a number of unusual properties, including high dipole moment (0.455 D) for a hydrocarbon, high reactivity toward addition reactions, driven by the 26 K. Cal/mole reduction in strain energy upon conversion, a tendency to complex with metals, and ring-opening reactions which sometimes involve vinylcarbenes as the reactive organic intermediates. The main impetus that led to the discovery of cyclopropenoid fatty acids came from the food and agricultural industries (3).

Pithecollobium dulce belongs to the Leguminosae plant family and is a medium-sized, evergreen, spiny tree about 18–20 m high. It is a native of tropical America and is cultivated throughout India. The pods are twisted, constricted and reddish-brown, and seeds are enveloped with pink to whitish pulp (4). The bark is used as a febrifuge or antipyretic (5). An exhaustive survey of the literature reveals that no work has been published about the seeds of *P. dulce*. The present report describes the unique occurrence of vernolic acid and cyclopropenoid fatty acids (malvalic acid and sterculic acid) along with the normal fatty acids in the seed oil of *P. dulce*.

EXPERIMENTAL PROCEDURES

The air-dried seeds of *P. dulce* were powdered and extracted thoroughly with light petroleum ether (b.p. 40–60°C) in a Soxhlet extractor for 24 h to yield 16.0% oil. Oil analyses were carried out according to the standard American Oil Chemists' Society (AOCS) method (6) and are listed in Table 1. The seed oil responded to the Halphen test (7) and picric acid thin-layer chromatography (TLC) test (8), thereby indicating the probable presence of cyclopropenoid and epoxy fatty acids, respectively. The seed oil did not respond to the 2,4-dinitrophenyl hydrazine (2,4-DNPH) TLC test (9) and the direct TLC test (10), thereby indicating the absence of keto and hydroxy fatty acids, respectively. Durbetaki titration (11) of the seed oil at two different temperatures (3 and 55°C) indicated 10.2% of epoxy and 5.3% of cyclopropenoid

TABLE 1
Analytical Values of *Pithecollobium dulce* Seed Oil

Oil content in seeds	16.0%
Unsaponifiable matter	2.1%
Iodine value	84.0
Saponification value	200.0
Halphen test	+[a]
Picric-acid TLC test	+
Direct TLC test	–
2,4-Dinitrophenyl hydrazine TLC test	–
Durbetaki titration at 3°C	10.2%
Durbetaki titration at 55°C	5.3%

[a]+, Positive response to the test; –, negative response to the test; TLC, thin-layer chromatography.

fatty acids, respectively. The infrared spectrum of the seed oil and its methyl esters showed the characteristic absorption bands at 1010 cm^{-1} and 825 cm^{-1} for the presence of cyclopropenoid and epoxy functional groups, respectively.

Acetolysis of epoxide. A portion of the oil (20.0 g) was stirred overnight at room temperature (27°C) with 80 mL of 10% (wt/vol) sulfuric acid in 200 mL glacial acetic acid as described by Wilson *et al.* (12). The acetolyzed product was diluted with distilled water and extracted with ether. The ether extract was washed thoroughly with distilled water and dried over anhydrous sodium sulfate. The solvent was removed in a stream of nitrogen.

Isolation of epoxy fatty acid (as dihydroxy fatty acid). For saponification, the acetolyzed product was treated with 0.8N alcoholic potassium hydroxide at room temperature (27°C). After careful acidification to pH 5 with 0.5N sulfuric acid, the liberated mixed fatty acids were extracted with ether. The ether extract was washed thoroughly with distilled water until neutral, and the solvent was removed in a stream of nitrogen. The separation of fatty acids into oxygenated and nonoxygenated fractions was accomplished by preparative TLC techniques. These fractions were examined for characterization of individual fatty acids. The yield of dihydroxy fatty acid was 10.0%. A concentrate of pure dihydroxy fatty acid (9.9%) was obtained by column chromatographic techniques.

Preparation of cyclopropenoid fatty acids derivatives. The nonoxygenated fraction (200 mg) was esterified by the Fischer esterification method and treated with 60 mL of absolute methanol saturated with silver nitrate (13). The reaction was carried out at room temperature with constant stirring for about 24 h. The ether extracts were dried over anhydrous sodium sulfate. The solvent was removed in a stream of nitrogen. The normal methyl esters and the ether and ketone derivatives of cyclopropenoid fatty acids were submitted to gas–liquid chromatographic analysis with the methyl esters of *Sterculia foetida* as a reference standard under similar conditions. The gas–liquid chromatography (GLC) instrument recorded directly the weight percent of individual peaks, which were identified and characterized by comparing their retention times with those of standard reference samples under similar conditions. The results are summarized in Table 2.

Instrumentation. The ultraviolet (UV) spectra were taken on a Hitachi 270-30 instrument (Tokyo, Japan) at 0.001% concentration in methanol. The infrared spectra were recorded on a Fourier transform infrared Bomem Michelson Series instrument as liquid films. The ^1H nuclear magnetic resonance (NMR) spectra were recorded on a Varian T-60 instrument (Palo Alto, CA) with $CDCl_3$ as solvent. The chemical shifts (δ) were measured in parts per million (ppm) downfield from the internal standard TMSi at δ = 0. The mass spectra were recorded on a Finnigan Mat (San Jose, CA) with a POP Micro Computer 810 at 70 eV with a source temperature 150°C. The GLC analysis was carried out on a Perkin-Elmer (Norwalk, CT) Model Sigma Unit with a 15% diethylene glycol succinate column on Chromosorb W, (250–354 μm) 45–60 mesh. The temperatures at injection port, detector port and oven were at 240, 240, and 190°C, respectively. The nitrogen flow and chart speed were 30 mL/min and 1 cm/min, respectively. The machine recorded directly the weight percent of individual peaks. The peaks were identified by comparing their retention times with those of standard reference samples under similar conditions. The melting points were recorded on a Thomas–Hoover capillary melting apparatus.

Chromatographic methods. The chromatographic methods have been described. Analytical TLC was performed on glass plates coated with 0.25- or 1.0-mm layers of silica gel G with 20 or 30% diethyl ether in hexane as the solvent system. Preparative TLC was effected on 20 cm × 20 cm plates with 1.0-mm layers of silica gel. When the plates were sprayed with 0.2% ethanolic solution of 2′,7′-dichlorofluoroscein, the separated bands were clearly visible under UV light. The fatty acids were extracted from the silica with ether. Column chromatography was performed with activated neutral alumina with ether as the eluting solvent.

TABLE 2
Component Fatty Acids of *Pithecollobium dulce* Seed Oil

Fatty acids	Percentage
Palmitic	12.1
Stearic	4.2
Behenic	10.6
Oleic	34.1
Linoleic	23.8
Vernolic (12,13-epoxy-octadec-*cis*-9-enoic acid)	10.0
Malvalic [7-(2-octacyclopropen-1-yl)heptanoic acid]	3.2
Sterculic [8-(2-octacyclopropen-1-yl)octanoic acid]	2.0

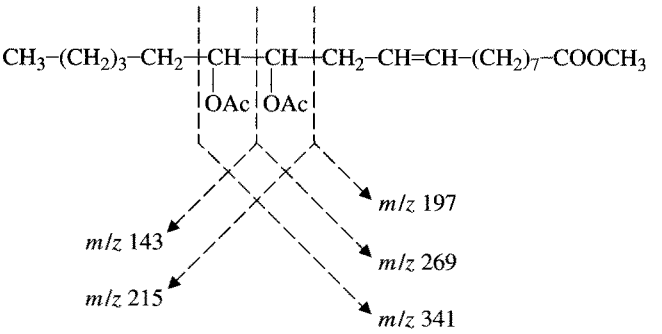
RESULTS AND DISCUSSION

The infrared spectrum of unsaturated dihydroxy fatty ester showed the characteristic absorption bands at 3450 and 1740 cm^{-1} for the presence of hydroxyl and ester carbonyl functional groups, respectively. The infrared spectrum also showed absorption bands at 715 and 1620 cm^{-1} for the presence of *cis* double bonds. However, the UV and infrared spectra showed no evidence for *trans* unsaturation or the presence of conjugation. The unsaturated dihydroxy acid, upon hydrogenation (14), gave 12,13-dihydroxy-octadecanoic acid, m.p. and mixed m.p. 96–97°C (literature m.p. 95–96°C). The unsaturated dihydroxy acid was cleaved with the permanganate–periodate reagent (15). GLC analysis of the resulting products as their methyl esters showed that the cleavage fragments were hexanoic acid (*p*-bromophenacyl ester, m.p. and mixed m.p. 70–71°C) and azelaic acid, m.p. and mixed m.p. 106–107°C (*p*-bromophenacyl ester, m.p. and mixed m.p. 131–132°C).

The unsaturated dihydroxy acid had the same R_1 value as *threo*-12,13-dihydroxy-octadec-*cis*-9-enoic acid obtained by acetolysis of *Vernonia anthelmintica* seed oil.

The ^1H NMR spectrum of unsaturated dihydroxy methyl ester gave signals at δ 5.46 (*m*, 2H, –C<u>H</u>=C<u>H</u>–), 3.66 (*s*, 3H,

$-COOCH_3$), 3.35 [*m*, 4H, 2H(–CH–O–) + 2H(–CH–OH)], 2.24 (*m*, 2H, $-CH_2-COO^-$), 2.0 (*m*, 4H, protons to the double bond $-CH_2-CH=CH-CH_2-$), 1.3 (*s*, 18H, $-(CH_2)_9-$ shielded methylene protons), and 0.88 (*t*, 3H, terminal $-CH_3$). After shaking with D_2O, the signal at δ 3.35 was reduced and integrated for the two protons only (*m*, 2H, –CH–O–), indicating that the hydroxyl protons signal was merged with the signal of –CH–O–. The mass spectrum of the diacetyl derivative of the unsaturated dihydroxy methyl ester showed a small molecular ion peak at *m/z* 412. The allylic cleavage (*m/z* 197) established the double bond at C_9 and C_{10}. The alpha cleavage on either side of the two acetate groups gave signals at *m/z* 341, 269, 215, and 143 and placed the two acetate groups at C_{12} and C_{13} (Scheme 1).

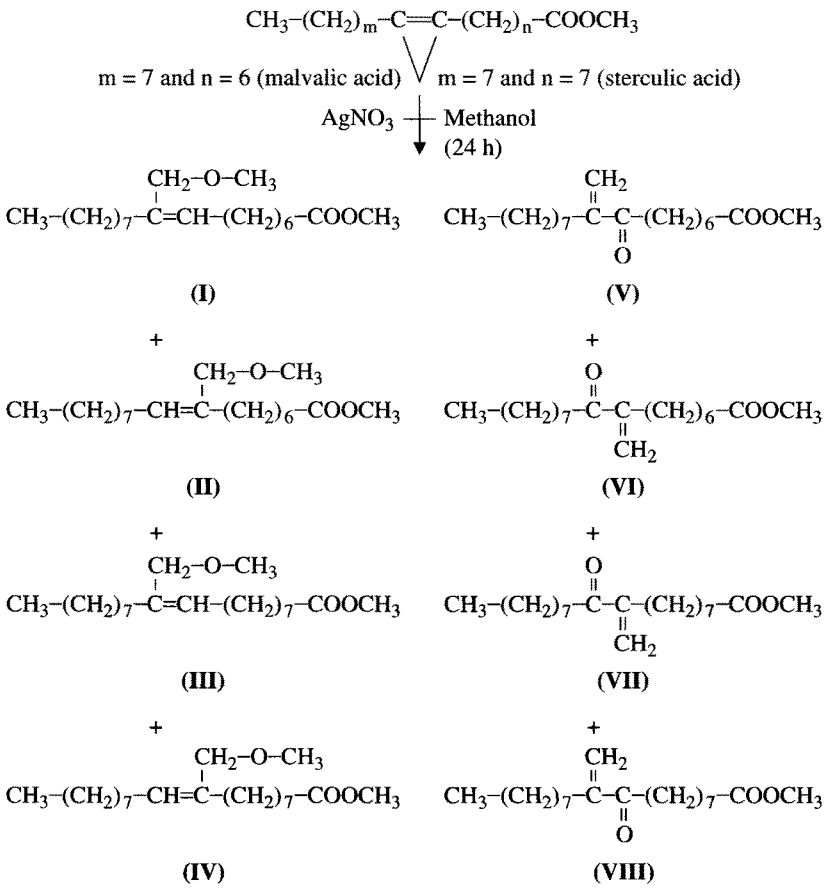


SCHEME 1

Thus, the structure of the isolated epoxy fatty acid, obtained as dihydroxy fatty acid, from *P. dulce* seed oil has been characterized as 12,13-epoxy-octadec-*cis*-9-enoic acid (vernolic acid).

The cyclopropenoid fatty acid characterization was determined by their ether and ketone derivatives as given in Scheme 2 (Structures **I–VIII**). The cyclopropenoid fatty acids and normal fatty acids were converted into their methyl esters by Fischer esterification. These fatty esters were again converted into their ether and ketone derivatives by treatment with an excess of absolute methanol saturated with silver nitrate. These ether and ketone derivatives were submitted to GLC analysis along with the normal fatty esters and compared with the derivatives of *S. foetida* seed oil as the reference standard. The individual peaks were recorded directly by weight percent and were identified by comparing their retention times with those of standard reference samples under similar conditions.

Thus, GLC analysis of the ether and ketone derivatives of the cyclopropenoid fatty acids characterized them as 7-(2-



SCHEME 2

octacyclopropen-1-yl)heptanoic acid (malvalic acid) and 8-(2-octacyclopropen-1-yl)octanoic acid (sterculic acid).

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