SHORT COMMUNICATIONS

Vernolic Acid in *Tabebuia argentia* Seed Oil: A Moderate Source of Oil

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***Tabebuia argentia*, Britt, Syn. *Tecoma argentia*, Burr and Schum, belonging to the Bignoniaceae plant family, contains palmitic (21.7%), stearic (3.8%), oleic (9.8%), linoleic (52.7%), linolenic (3.0%) and vernolic (9.0%) acids. These fatty acids were characterized by infrared (IR), nuclear magnetic resonance (NMR), mass spectrometry (MS), gas-liquid chromatography (GLC) and chemical degradations.**

KEY WORDS: Bignoniaceae, 12,13-epoxy-*cis*-octadec-9-enoic acid (vernolic acid), fatty acids, seed oil, *Tabebuia argentia*, syn. *Tecoma argentia*.

Seed oils rich in epoxy acids are of potential interest as stabilizers in plastic formulations (1) and in the preparation of other long-chain compounds (2). Epoxy compounds have also attracted much attention owing to their co-carcinogenic properties (3). *Tabebuia argentia* is a moderate source of oil and shows sufficient promise for its exploitation as an alternative source of commercial oil. New and interesting unusual fatty acids present in high concentration in certain seed oils are being exploited for commercial use (*e.g. Vernonia anthelmintica* and *roxburghii* seed oils). These acids of unusual structures are highly important to the chemical industry as raw material for the production of oleochemicals (4).

Tabebuia argentia belongs to the Bignoniaceae plant family, which consists of 105 genera and more than 550 species (5). It is a moderate-size deciduous tree found in the warmer regions of India. It is often cultivated in gardens as an ornamental plant for its beautiful yellow flowers (6).

The present paper describes the occurrence of vernolic acid along with other usual fatty acids. This is the first report on this seed oil.

EXPERIMENTAL PROCEDURES

The air-dried seeds were powdered and extracted thoroughly with light petroleum ether (b.p. 40–60°C) in a Soxhlet apparatus to yield 24.0% of oil. An infrared spectrum of the oil and its methyl esters showed weak absorption band at 825 cm^{-1} due to an epoxy functional group. The oil gave a positive picric-acid thin-layer chromatography (TLC) test (7), indicating the presence of epoxy fatty acids. However, the oil did not give positive 2,4-dinitrophenylhydrazine (DNPH) thin-layer chromatography (TLC) (8) and Halphen test (9), indicating the absence of oxo and cyclopropenoid fatty acids, respectively. The direct TLC of the oil revealed the absence of hydroxy fatty acids when using castor oil as standard reference. The Durbetaki titration (10) of oil at 3°C indicated 9.2% epoxy fatty acids. The analytical values for the oil so obtained were determined according to (AOCS) American Oil Chemists' Society (11) methods and are listed in Table 1.

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TABLE 1

Analytical Data of *Tabebuia argentia* Seed Oil[a]

Oil content	24.0%
Unsaponifiable matter	2.9%
Iodine value	116.3
Saponification value	203.1
Halphen test	−
Picric-acid TLC test	+
2,4-DNP TLC test	−
HBr equivalent at 3°C	9.2%
Infrared (IR)	825 cm^{-1}

[a]+ Indicates positive response to the test; − indicates negative response to the test.

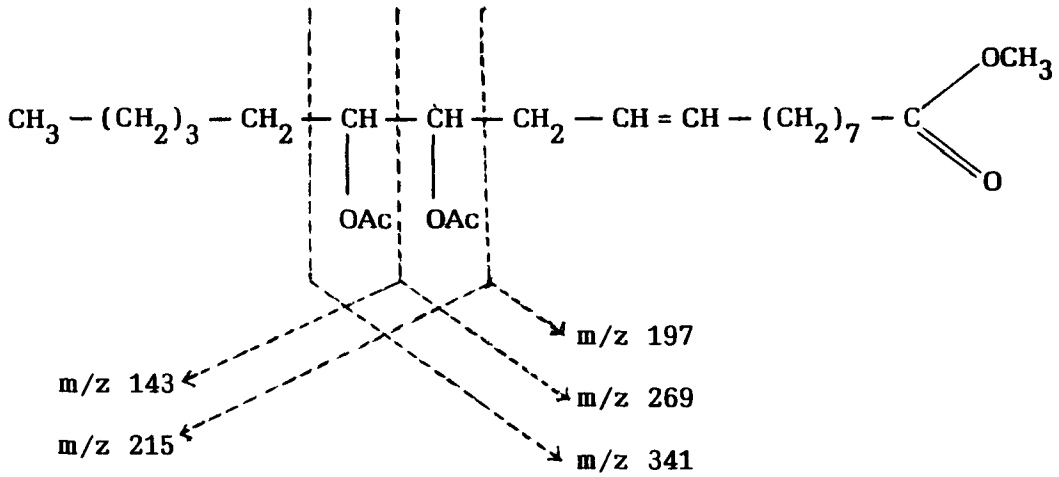
Acetolysis of the opoxide group was affected by treatment of the oil (20 g) with glacial acetic acid and 10% sulphuric acid (5:2 v/v) at room temperature following the procedure of Wilson *et al.* (12). The acetolyzed product was saponified by stirring overnight with 0.8N alcoholic potassium hydroxide at room temperature. The non-saponifiable matter was removed. After careful acidification to pH 5 with 0.5N sulphuric acid, the mixed fatty acids were extracted immediately with ether. The ether solution was washed with distilled water until neutral. The solvent was removed in a stream of nitrogen. Separation of these mixed fatty acids into oxygenated and non-oxygenated fractions was accomplished by preparative TLC (petroleum ether and ether 7:3 v/v), and these fractions were examined for characterization of individual fatty acids. The yield of corresponding dihydroxy acid was equivalent to 9.0% of the total oil.

The methyl esters were prepared by transesterification with absolute methanol containing 1% sodium methoxide. The reaction was allowed to proceed by refluxing the solution for 30 min, and the methyl esters were extracted with ether. The ether solution was washed with distilled water and dried over anhydrous sodium sulphate. The solvent was removed in a stream of nitrogen.

Infrared (IR) spectra were taken on a Hitachi 270-30 Model instrument (Hitachi Co., Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra were recorded on a Varian T-60 Model instrument (Varian Associates, Palo Alto, CA) with tetramethylsilane as internal standard. Mass spectra were recorded on a Jeol-JMS-D-300 Model instrument (Jeol Ltd., Akishima, Japan). Gas-liquid chromatography (GLC) analyses were carried out on a Perkin-Elmer Sigma Unit (Perkin-Elmer, Nowalk, CT) with a stainless-steel column coated with 15% diethyleneglycol succinate (DEGS) on chromosorb W, 45–60 mesh.

RESULTS AND DISCUSSION

The infrared spectrum of the dihydroxy ester isolated by acetolysis of the epoxy acid had a strong absorption band at 3450 cm^{-1} for hydroxyl functional group. The un-



SCHEME 1

TABLE 2

Component Acids of *Tabebuia argentia* Seed Oil

Fatty acids	Percentage
Palmitic	21.7
Stearic	3.8
Oleic	9.8
Linoleic	52.7
Linolenic	3.0
Vernolic	9.0

saturated dihydroxy acid upon hydrogenation (13) furnished 12,13-dihydroxyoctadecanoic acid (14), m.p. 96–97°C (lit. m.p. 95–96°C). The unsaturated dihydroxy acid was cleaved with permanganate-periodate reagent (15). GLC analysis of the resulting products as their methyl esters showed that the cleavage fragments were hexanoic and azelaic acids when *Cassia siamea* (16) seed was used as standard reference.

The unsaturated dihydroxy acid had the same R_f value as *threo*-12,13-dihydroxy oleic acid obtained by acetolysis of *Vernonia anthelmintica* seed oil.

The NMR spectrum of the unsaturated dihydroxy ester gave signals at δ 5.46 (2H, −CH=CH−), 3.66 (3H, $-COOCH_3$), 3.35 (4H, 2H(−CH−O−) + 2H−(OH), 2.24 (2H, α — to the carbonyl function), 2.9 (protons to the double bond), 1.3 (shielded methylene protons), 0.88 (3H, terminal $-CH_3$). After shaking the sample with D_2O, the signal at δ 3.35 was reduced and integrated for two protons only (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 fatty 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). Alpha cleavage on either side of two acetate groups gave signals at *m/z* 341, 269, 215, 143 and placed the acetate groups at C(12) and C(13) (Scheme 1).

Thus, the epoxy fatty acid was characterized as 12,13-epoxy-*cis*-octadec-9-enoic (vernolic) acid.

Tabebuia argentia seed oil thus contains a minor amount of vernolic acid (9.0%). This seed oil also contains palmitic (21.7%), stearic (3.8%), oleic (9.8%), linoleic (52.7%) and linolenic (3.0%) acids. The results are summarized in Table 2.

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