SHORT COMMUNICATION

Unusual Fatty Acids in *Brunfelsia americana* Seed Oil: A Rich Source of Oil

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***Brunfelsia americana* Linn, belonging to the Solanaceae plant family, was found to contain ricinoleic acid together with cyclopropenoid and normal fatty acids. These fatty acids were characterized by infrared, nuclear magnetic resonance, mass spectrometry, gas-liquid chromatography techniques and chemical degradations.**

KEY WORDS: ***Brunfelsia americana*, fatty acids, malvalic, rare occurrence, ricinoleic, seed oil, Solanaceae, sterculic.**

Seed oils containing hydroxy fatty acids are industrially important as they are used in protective coatings, plastics and a variety of synthetic intermediates. Cyclopropenoid fatty acids have attracted much attention owing to their biological effects on animals (1-3) and their co-carcinogenic properties (4,5).

Brunfelsia americana is a free flowering shrub, native of tropical America. It is cultivated in gardens as an ornamental plant. Flowers are yellow with a pleasant odor (6). An exhaustive survey of literature reveals that no work has been reported on the seeds of *Brunfelsia americana*. The present study describes the rare occurrence in minor amounts of ricinoleic acid and cyclopropenoid fatty acids with normal fatty acids in *Brunfelsia americana* seed oil.

EXPERIMENTAL PROCEDURES

The air-dried seeds of *Brunfelsia americana* were powdered and extracted thoroughly with light petroleum ether (b.p. 40-60°C) in a Soxhlet extractor for 24 hours to yield 30.0% oil. The ether extracts were dried over anhydrous sodium sulphate and the solvent was removed *in vacuo* at 40°C. The physicochemical characteristics of oil obtained were determined according to AOCS (7) methods and are listed in Table 1.

The oil responded to the Halphen (8) test, thereby indicating the probable presence of cyclopropenoid fatty acids. However, the oil did not respond to picric-acid (9) thin-layer chromatography (TLC) and 2,4-DNP (10) TLC tests, thereby indicating the absence of epoxy and oxo fatty acids, respectively. The direct TLC of oil revealed the presence of hydroxy fatty acids when castor oil was used as standard reference. The Durbetaki titration (11) of oil at 55°C indicated 2.6% of total cyclopropenoid fatty acids.

Infrared (IR) spectra of the oil and its methyl esters showed characteristic bands at 1010 cm^{-1} and 3450 cm^{-1} for cyclopropenoid and hydroxyl functional groups, respectively. The methyl esters of the oil had a nuclear magnetic resonance (NMR) signal typical for cyclopropene hydrogens at δ 0.72. Saponification of the oil was affected by stirring overnight at room temperature with 0.8N alcoholic potassium hydroxide. The non-saponifiable matter was removed. The mixed fatty acids were recovered by ether extraction and were partitioned according to Gunstone's method (12) between petroleum ether and 20% aqueous methanol. The yield of hydroxy fatty acid was 5.0%. A concentrate of pure hydroxy fatty acid (4.9%) was obtained by preparative TLC.

TABLE 1

Physicochemical Characteristics of *Brunfelsia americana* Seed Oil

Oil content	30.0%
Unsaponifiable matter	2.2%
Iodine value	124.0
Saponification value	198.0
Halphen test	+
Picric acid TLC test	−
2,4-DNP TLC test	−
HBr equivalent at 55°C	2.6%
Infrared (IR)	1010 cm^{-1}
	3450 cm^{-1}
Nuclear magnetic resonance	δ 0.72

+ Indicates positive response to the test.
− Indicates negative response to the test.

IR spectra were taken as liquid films on a Hitachi Model 270-30 (Hitachi Co., Tokyo, Japan) instrument. NMR spectra were recorded on a Varian T-60 Model (Varian Associates, Palo Alto, CA) instrument. Chemical shifts (δ) were measured in ppm downfield from internal tetramethylsilane. Gas-liquid chromatography (GLC) analysis was carried out on a Perkin-Elmer Model Sigma Unit (Perkin-Elmer, Norwalk, CT) with 15% DEGS stainless steel column (2 m × 3 mm) on chromosorb W, 45-60 mesh. The temperature of the injection port, detector port and oven were 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.

RESULTS AND DISCUSSION

Oxygenated fatty acid. The hydroxy acid showed a strong infrared absorption band at 3450 cm^{-1} for free hydroxyl functional group and absorption at 715 cm^{-1} and 1620 cm^{-1} for the presence of a *cis* double bond. The unsaturated hydroxy fatty acid on oxidation with potassium permanganate in acetic acid (13) gave azelaic acid, m.p. 106-107, and heptanoic acid (*p*-bromophenacyl ester m.p. 66-67°C).

The NMR spectrum of the hydroxy acid methyl ester exhibited signals at δ 5.4 (2H, -CH=CH-), 3.6 (3H, $-COOCH_3$), 3.3 (1H, -*CH*OH), 2.75 (1H, -CH-O*H*), 2.2 (6H, overlapping signals ascribable to allylic protons and protons α- to the carbonyl), 1.2 (chain, $-CH_2-$) and 0.88 (3H, terminal $-CH_3$). After shaking with D_2O, the signals at δ 2.75 disappeared with a small change in the signal at δ 3.3.

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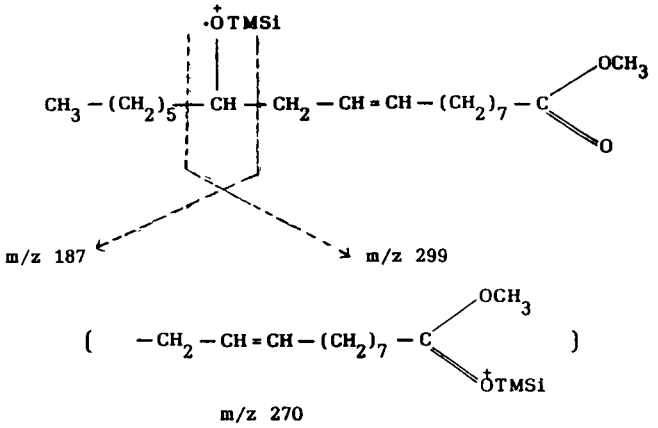

SCHEME 1

TABLE 2

Component Acids of *Brunfelsia americana* Seed Oil

Fatty acids	Percentage
Myristic	2.1
Palmitic	9.7
Stearic	5.0
Oleic	16.9
Linoleic	58.8
Ricinoleic	5.0
Malvalic	1.1
Sterculic	1.4

The mass spectrum of the trimethylsilyl (TMSi) derivative of hydroxy olefinic ester was identical to the TMSi derivative of authentic methyl ricinoleate. The structure-revealing ions were observed at *m/z* 187 and 299 and a TMSi rearrangement (14) ion at *m/z* 270 unequivocally established the position of the hydroxy group at C (12) and indicated the double bond at C (9) (Scheme 1).

Non-oxygenated fatty acids. The transesterified methyl esters of non-oxygenated fatty acids (200 mg) were treated with 60 mL of absolute methanol saturated with silver nitrate (15). The reaction was allowed to proceed at room temperature with stirring for 24 hours. The normal methyl esters and the reaction products from cyclopropenoid fatty esters were recovered from the reaction mixture by adding 100 mL of distilled water and extracting them with ether. The ether extracts were dried over anhydrous sodium sulphate and the solvent was evaporated in a stream of nitrogen. The GLC analysis was carried out with *Plumbago zeylanica* (16) esters as a reference standard.

The seed oil of *Brunfelsia americana* was found to contain ricinoleic acid (5.0%) and cyclopropenoid fatty acids, such as malvalic (1.1%) and sterculic (1.4%). Palmitic acid (9.7%) is the major component amongst the saturated acids, with smaller amounts of myristic (2.1%) and stearic (5.0%). The unsaturated fatty acids are oleic (16.9%) and linoleic (58.8%). The results are summarized in Table 2.

ACKNOWLEDGMENT

One of the authors (KMH) is indebted to the Council of Scientific and Industrial Research, New Delhi, for the award of a Senior Research Fellowship.

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[Received August 23, 1990; accepted February 28, 1991]