Novel Fatty Acids in *Azima tetracantha* Seed Oil

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***Azima tetracantha* Lam, belonging to the Salvadoraceae plant family, was found to contain ricinoleic acid (9.8%) and cyclopropenoid fatty acids (9.6%) along with normal fatty acids.**

KEY WORDS: *Azima tetracantha*, fatty acids, malvalic, ricinoleic, Salvadoraceae, seed oil, sterculic.

Azima tetracantha is a rambling spinous shrub flowering throughout the year in India. The juice of the leaves is said to relieve the cough of phthisis and asthma. It is a good hedge plant. The berries are edible (1).

An exhaustive survey of literature reveals that no work has been reported on the seeds of *Azima tetracantha*. The present investigation describes the unusual occurrence of ricinoleic acid and cyclopropenoid fatty acids along with the normal fatty acids in *Azima tetracantha* seed oil as well as in the Salvadoraceae plant family.

EXPERIMENTAL PROCEDURES

The air-dried seeds of *Azima tetracantha* were powdered and extracted thoroughly with light petroleum ether (b.p. 40–60°C) in a Soxhlet extractor for 24 hr. The ether extracts were dried over anhydrous sodium sulphate, and the solvent was removed *in vacuo* at 40°C. The analytical characteristics of oil so obtained were determined according to AOCS methods (2) and are listed in Table 1.

The oil responded to the Halphen (3) test, indicating the probable presence of cyclopropenoid fatty acids. However, the oil did not respond to picric-acid (4) thin-layer chromatography (TLC) and 2,4-DNP (5) TLC tests, indicating the absence of epoxy and keto fatty acids, respectively. Direct thin-layer chromatography of oil revealed the presence of hydroxy fatty acids when using castor oil as reference standard. The Durbetaki titration (6) of oil at 55°C indicated 9.8% 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 0.8 N alcoholic potassium hydroxide. The non-saponifiable matter was removed. The mixed fatty acids (20 g) were recovered by usual ether extraction and were partitioned according to Gunstone's method (7) between petroleum ether and 80% methanol. The yield of hydroxy fatty acid (1.96 g) was obtained from aqueous methanol (9.8%). A concentrate of pure hydroxy fatty acid (9.7%) was obtained by preparative TLC.

IR spectra were taken as liquid films on a Hitachi 270-30 Model Instrument (Tokyo, Japan). The NMR spectra were recorded on a Varian T-60 Model instrument (Palo Alto, CA). The chemical shifts (δ) were measured in ppm downfield from internal tetramethylsilane. The mass spectrum was recorded on a JEOL-JMS-D-300 Model instrument (Tokyo, Japan). Gas-liquid chromatography (GLC) analysis was carried out on a Perkin-Elmer Model Sigma Unit (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.

TABLE 1

Analytical Data of *Azima tetracantha* Seed Oil

Oil content	12.0%
Unsaponifiable matter	2.3%
Iodine value	141.0
Saponification value	201.5
Halphen test	+[a]
Picric acid TLC test	−[a]
2,4-DNP TLC test	−
HBr equivalent at 55°C	9.8%
Infrared (IR)	1010 cm^{-1}
	3450 cm^{-1}
Nuclear magnetic resonance (NMR)	δ 0.72

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

RESULTS AND DISCUSSION

Oxygenated fatty acid. The hydroxy fatty acid showed a strong infrared absorption band at 3450 cm^{-1} for free hydroxyl functional groups 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 (8) gave azelaic acid, m.p. 106–7°C and heptanoic acid (*p*-bromophenacyl ester, m.p. 66–7°C).

The NMR spectrum of the hydroxy acid methyl ester exhibited signals at δ 5.4 (2H, -C*H*=C*H*-), 3.6 (3H, -COOCH_3), 3.3 (1H, -C*H*OH), 2.75 (1H, -CH-O*H* which disappeared on addition of D_2O), 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).

The mass spectrum of the trimethyl silyl (TMSi) derivative of the 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 (9) ion at *m/z* 270. These results suggest the position of the hydroxy group at C(12) with the double bond at C(9) (Scheme 1).

Thus, the structure of hydroxy fatty acid has been

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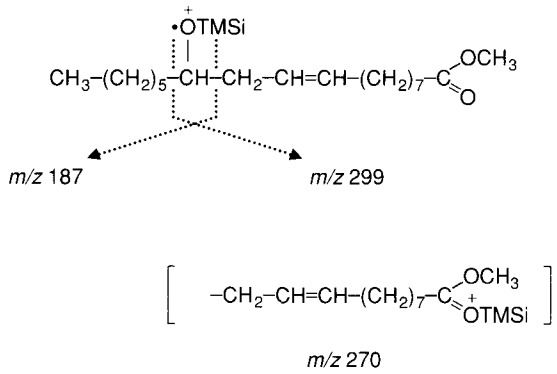

SCHEME 1

TABLE 2

Fatty Acid Composition of *Azima tetracantha* Seed Oil

Fatty acids	Percentage
Lauric	3.5
Myristic	4.2
Palmitic	5.2
Stearic	1.6
Oleic	15.3
Linoleic	28.8
Linolenic	22.0
Ricinoleic	9.8
Malvalic	4.0
Sterculic	5.6

characterized as 12-hydroxy-*cis*-octadec-9-enoic (ricinoleic) acid.

Nonoxygenated fatty acids. The transesterified methyl esters of nonoxygenated fatty acids (200 mg) were treated with 60 mL of absolute methanol saturated with silver nitrate (10). The reaction was allowed to proceed at room temperature with stirring for 24 hr. 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* (11) esters as reference standard for cyclopropenoid fatty acids.

The fatty oil of *Azima tetracantha* contains ricinoleic (9.8%), malvalic (4.0%) and sterculic (5.6%) as its unusual fatty acids. The other component fatty acids are given in Table 2.

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