Ricinoleic Acid in *Phyllanthus niruri* Seed Oil[1]

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ABSTRACT

Seed oil of *Phyllanthus niruri* (Euphorbiaceae) contains 1.2% ricinoleic (12-hydroxy-*cis*-9-octadecenoic) acid, previously unknown in the genus *Phyllanthus*. Identification is based on thin layer and gas liquid chromatography, infrared, nuclear magnetic resonance, and mass spectrometry analysis as well as chemical methods. Other major components of the oil are linoleic acid (21%) and linolenic acid (51.4%).

INTRODUCTION

Phyllanthus niruri L. is a member of the family Euphorbiaceae, distributed throughout the arid regions of India, Ceylon and most tropical countries, but not Australia. The plant is annual, up to 2 ft high, and is used as a diuretic by the natives of India (1). Seeds are trigonous and rounded on the back with parallel longitudinal ribs.

The fatty acids of *Phyllanthus* species (2,3) were believed to be mixtures of linolenic, linoleic, oleic and saturated acids. Linolenic acid represents ca. 35% of the total fatty acid mixture and thus these oils are classified as "linolenic-rich" oils. In addition, the Euphorbiaceae family is somewhat exceptional in possessing a few species which elaborate unusual acids in the seed fats; one of the most important ones is ricinoleic acid in the *Ricinus* species.

A search of the literature failed to show the fatty acid composition of *Phyllanthus niruri* seed oil. This study was undertaken to determine the fatty acid composition and to confirm the presence or absence of a hydroxy acid in this seed oil.

MATERIALS AND METHODS

Ground seeds were exhaustively Soxhlet-extracted with hexane, and the solvent was evaporated under vacuum in a rotary evaporator. The analytical values of oil and seeds (Table I) were determined according to the procedures recommended by the AOCS (4). Infrared (IR) spectra were recorded from liquid films on sodium chloride disks or 1% $CHCl_3$ solution using a Perkin-Elmer Model 621 spectrophotometer, and ultraviolet (UV) measurements were made on a methanolic solution with a Beckman Model DK-2A spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded from deuteriochloroform solutions with a Varian Model A60 spectrometer. Chemical shifts were measured in ppm downfield from internal tetramethylsilane (δ=0). The abbreviations s, d, m, br and t denote singlet, doublet, multiplet, broad and triplet, respectively. Mass spectra (MS) were measured with an AEI MS-9 mass spectrometer. Gas liquid chromatography (GLC) of the silylated methyl esters was completed with a Perkin-Elmer Model 154 equipped with a thermal conductivity detector, using a stainless steel packed column (2 m x 3 mm) coated with diethylene glycol succinate (DEGS, 15% on Chromosorb W, 45-60 mesh). The separations were done isothermally at 200 C. Melting points were observed on a Kofler apparatus and are uncorrected.

Thin layer chromatography (TLC) was performed on plates coated with 0.25-mm or 1.0-mm layers of Silica Gel G with hexane/ether/acetic acid (70:30:1, v/v/v) as the developing solvent. Argentation TLC was effected on Silica Gel G impregnated with 12% silver nitrate. The spots on analytical TLC plates were visualized by charring with a 20% aqueous solution of perchloric acid.

The isolation of the fatty acids from the oil, methylation of the mixed fatty acids, purification of hydroxy ester and hydrogenation were done as previously described (5). Silylation of the methyl ester was done by treating with hexamethyldisilazane and trimethyl chlorosilane (6). Deoxygenation of the saturated hydroxy ester and permanganate-periodate cleavage of the original hydroxy acid were done according to published procedure (7,8). The GLC identification of the cleavage products was done after methylation with ethereal diazomethane (9).

RESULTS AND DISCUSSION

Seeds from the genus *Phyllanthus* (2,3) have not been shown to contain ricinoleic acid. A screening program of seed oils containing unusual fatty acids, with particular reference to oxygenated fatty acid (5,10-12), revealed that the oil of *Phyllanthus niruri* contains an oxygenated acid as a minor component. The IR spectra of the oil, as well as its methyl ester, showed a hydroxyl band at 3450 cm^{-1}. The UV spectra showed the absence of conjugation in the component acids. TLC analysis of the methyl esters showed two classes of components with R_f values indicating normal methyl esters and a monohydroxy methyl ester. The hydroxy ester was isolated by preparative TLC and subsequently purified by silica gel column chromatography. IR of the isolated ester again showed the hydroxyl band and the absence of any *trans* unsaturation (965 cm^{-1}). The NMR spectrum exhibited signals at δ 5.4m (2H,-C<u>H</u>=C<u>H</u>), 3.6s (3H, COOC<u>H</u>$_3$), 3.3br (1H,-C<u>H</u>-OH), 2.75br (1H, -CH-O<u>H</u>, disappeared upon addition of D_2O), 2.2 (6H, overlapping signals ascribable to allylic protons and the protons α to the carbonyl), 1.2 br, s (chain - C<u>H</u>$_2$), and 0.88t(3H, terminal - C<u>H</u>$_3$). After shaking with D_2O, the signal at δ 2.75 disappeared with a small change in the signal at δ 3.3. The acetate derivative of the pure hydroxy ester showed a strong band at 1235 cm^{-1} and no hydroxyl absorption at 3450 cm^{-1} in its IR spectrum. The NMR spectrum (acetate derivative) showed no unusual features

TABLE I

Analytical Data on *P. niruri* Seeds and Oil

Seeds	
Oil content (%)	15.7
Protein content (%)	16.8
Moisture (%)	5.3
Seed oil	
Iodine value (Wijs)	187
Saponification value	198
Refractive index, n_D^{40}	1.4885
Unsaponifiables (%)	4.0

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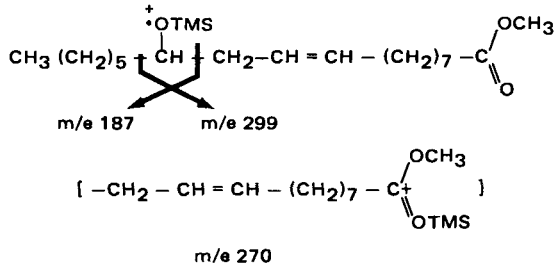
apart from two expected, but significant, signals at δ 4.7 m (1H, - CH - OAc), and 1.9s (3H, - $OCOCH_3$). The disappearance of the original signal for hydroxyl group confirmed the original acid as hydroxy acid.

The MS of the trimethylsilyl (TMS) derivative of the hydroxy-olefinic ester was identical to the TMS derivative of authentic methyl ricinoleate. Structure-revealing ions were observed at m/e 187 and 299, and a TMS rearrangement ion (13) at m/e 270 unequivocally established the position of hydroxyl at C-12 and indicated double bond at C-9.



The peaks corresponding to the trimethylsilyl ion, m/e 73 [$(CH_3)_3$ Si^+], and the rearranged ion, m/e 75 [HO = Si $(CH_3)_2$] were other major peaks of the spectrum. The fragment ions at m/e 369 (M-15), 353 (M-31) and 337 (M-47) were also observed as previously reported (13) in the spectra of TMS derivatives.

Catalytic hydrogenation (Pd/C) of hydroxy-olefinic ester gave a saturated hydroxy ester melting at 56-57 C depressed to 49-50 C upon mixing with methyl 9-hydroxystearate (mp 52-53 C). Reductive deoxygenation of methyl 12-hydroxystearate by hydrogen iodide-phosphorus followed by zinc and hydrochloric acid (7) furnished methyl stearate, as identified by GLC and co-TLC. This established a normal C-18 skeleton for the hydroxy acid.

Oxidative cleavage of the original hydroxy acid by permanganate-periodate (8) yielded two major fragments which were identified by GLC after methylation with diazomethane. Identified fragments were azelaic and 3-hydroxypelargonic acids. Comparision of the retention times with authentic samples demonstrated the identity of the structures. Formation of the azelaic acid showed that the double bond was at C-9 and that the hydroxyl function was not located between the double bond and the carboxyl group. The other product, 3-hydroxy pelargonic acid, placed the hydroxyl function at C-12.

On the basis of these physical and chemical evidences, the hydroxy acid was characterized as 12-hydroxy-*cis*-9-octadecenoic acid, commercially known as ricinoleic acid. The identity of this fatty acid was also confirmed by cochromatography with authentic ricinoleate. The total fatty acid composition of *P. niruri* oil is given in Table II.

TABLE II

Composition of Silylated Methyl Esters from *P. niruri* Seed Oil

Component	Area (% by GLC)
16:0	13.9
18:0	5.3
18:1	7.2
18:2	21.0
18:3	51.4
18:1-OH	1.2

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