The C_{16} Monoenoic Acid of *Zanthoxylum alatum* Seed Oil

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ABSTRACT

As a part of our screening program on unusual fatty acid-containing seed oils, we observed that the oil of *Zanthoxylum alatum* contains 15.4% of a C_{16} monoenoic acid. It was characterized as *cis*-9-hexadecenoic by the GLC analysis of oxidative cleavage fragments from an isolated monoene fraction.

INTRODUCTION

Zanthoxylum alatum, Roxb (Rutaceae) Vern. Tejbal, is a small tree or shrub, distributed throughout the outer Himalayas. All parts of the plant are pungently aromatic and fruits are used as herbal medicines and fish poison.

C_{16}-Monounsaturated acids are not as common in seed fats as those of C_{18} chain length. Among the C_{16} acids, the *cis/trans* monounsaturation has been reported at the 9,5,6,3 and 7 positions; these hexadecenoic acids usually are found in small quantities in seed fats (1-3). Spencer and coworkers (4) have recently reported the presence of 82.2% *cis*-6-hexadecenoic acid in the seed oil of *Thunbergia alata*. Raju and coworkers (5) have reported the presence of 15% *cis*-9-hexadecenoic acid in *Tricuspedaria lanceolata* seed oil.

In this paper we report the isolation and identification of a C_{16} monoenoic acid in the oil of *Zanthoxylum alatum*.

EXPERIMENTAL PROCEDURES

The seed oil of *Z. alatum* was obtained by 6-hr Soxhlet extraction of the ground seed with petroleum ether (40-60 C) and was subsequently neutralized by passing it in chloroform through a short column of alumina to remove the free fatty acids. Seed and oil properties were determined by AOCS methods (6). The fatty acids free from unsaponifiable matter were obtained by saponification with aq. ethanolic KOH. The mixed methyl esters prepared by acid-catalyzed methylation of mixed fatty acids were examined qualitatively by direct thin layer chromatography (TLC) for detecting oxygenated/nonoxygenated fatty acids. Argentation TLC (Ag^+/TLC) and reverse phase TLC procedures were used to detect the type of unsaturation and chain length of the component esters. Silica Gel G plates impregnated with 12% silver nitrate and petroleum ether/diethylether (7:3 v/v) were used for Ag^+/TLC. Siliconized silica Gel G plates and an acetonitrile/acetic acid/ water (70:10:20 v/v) system was used for reverse phase TLC. Individual ester fractions were isolated using preparative Ag^+/TLC. The bands were visualized by spraying the plates with a 5% alcoholic solution of 2',7'-dichlorofluorescein and viewing under ultraviolet (UV) light. The individual bands were scraped from the plates and extracted with diethyl ether.

Infrared (IR) spectra were obtained with a Perkin-Elmer 621 spectrophotometer in liquid film. UV measurements were made in methanolic solution with a Beckman DK-2A spectrophotometer. Methyl esters prepared by esterification with absolute methanol and 1% sulfuric acid were chromatographed on stainless steel packed column (2 m x 3 mm) coated with 15% DEGS or a (60 cm x 4 mm) column of 2% SE 30 on Chromosorb W (40-60 mesh) using a Perkin-Elmer model 154 gas liquid chromatograph. The separations were carried out isothermally at 200 C, chart speed 0.76 m/hr with a hydrogen flow of 70 ml/min. Identification of the peaks were made by comparison of their relative retention times with those of known esters of linseed oil and standard ester samples.

The permanganate-periodate oxidative cleavage of the unsaturated acids were performed following the Von Rudloff procedure (7). Cleavage products after esterification with diazomethane were analyzed by GLC using standard samples.

RESULTS AND DISCUSSION

The seed and oil characteristics of *Z. alatum* were: oil, 20%; iodine value (Wijs), 114; saponification value, 187; protein content (N x 6.25), 14.6%; moisture, 6.3%; refractive index, 1.4850 nD^{30}.

The UV and IR spectral analyses of the oil and its methyl ester showed no conjugation and *trans* absorption. Qualitative TLC indicated the presence of only the usual fatty acids. Ag^+/TLC of the methyl esters gave clear spots corresponding to the saturates, monoenes, diene and triene.

GLC analysis of methyl esters using nonpolar SE-30 column showed only C_{16} and C_{18} acids, whereas DEGS column showed 6 well-defined peaks in the chromatogram. The fatty acid composition of *Z. alatum* seed oil was: 16:0, 19.9%; 16:1, 15.4%; 18:0, 2.4%; 18:1, 22.7%; 18:2, 19.1% and 18:3, 20.3%.

A sample of the methyl ester was separated into 4 fractions by preparative Ag^+/TLC. The composition of each fraction is shown in Table I.

TABLE I

Fatty Acid Composition of *Z. alatum* Seed Oil
Methyl Ester Separated by Preparative $AgNO_3$-TLC

Fractions	Fatty acid composition by GLC
I (Saturated)	16:0 (89.2%) 18:0 (10.7%)
II (Monoene)	16:1 (40.4%) 18:1 (59.5%)
III (Diene)	18:2 (100%)
IV (Triene)	18:3 (100%)

Fraction I (22% by wt) was analyzed by GLC and found to be saturated esters. The monoenoic fraction II (38.1% by wt) showed no *trans* absorption. The presence of 16:1 (40.4%) and 18:1 (59.5%) acids was shown by GLC analysis. The position of the double bonds in the 2 monoenoic acids was established by Von Rudloff's (7) oxidative cleavage followed by GLC analysis of fragment esters. A GLC chromatogram showed that the C_7 and C_9 monocarboxylic ester fragments are equivalent to the proportion of 16:1 and 18:1 esters in fraction II. The only dicarboxylic acid detected was azelaic acid, the proportion of which doubles the content of monocarboxylic acids. These oxidative cleavage data established the presence of 9-hexa- and -octadecenoic acids in fraction II.

The diene and triene fractions (II, 19% and III, 20.3% by wt, respectively) showed no *trans* unsaturation. They

were identified as *cis*-9,12-octadecadienoic and *cis*-9,12,15-octadecatrienoic acid by Ag^+/TLC.

Although C_{16} monoenoic acid has been reported earlier (3) in 2 species of Rutaceae, the seed oil of *Z. alatum* appears to be the richest source of palmitoleic acid in Rutaceae seed oils reported so far.

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