☘*Vernonia volkameriaefolia* Seed Oil: A Rich Source of Epoxy Acid

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ABSTRACT

The seeds of *Vernonia volkameriaefolia* contain 20% oil and are a good source of epoxidized triglycerides. The vernolic acid (*cis*-12, 13-epoxy-*cis*-9,10-octadecenoic acid) content of the oil was 63.5%. The structure of this compound was established by chemical and physical methods, including a study of the mass spectral fragmentation of the parent compound and its derivatives.

INTRODUCTION

Seed oils of the Compositae have been found to contain many unusual fatty acids and considerable variation exists in the amounts of individual components found in such seeds. Oil from the seed of certain species in the genus *Vernonia* is of particular interest because of the occurence of large amounts of epoxy-containing acids. The interest in seed oils rich in epoxy acids has been stimulated by their potential as a replacement for synthetic epoxy compounds that are widely used as stabilizers for plastics formulation and in the preparation of other long-chain compounds. Among epoxy-containing seed oils, the most promising species appear to be *Vernonia anthelmintica, Stokesia laevis, Euphorbia lagascae* (1) and *Vernonia galamensis* (2). Continuing studies designed to identify new sources of epoxy oils, the seed oil of *Vernonia volkameriaefolia* has been examined and shown to be a rich source of vernolic acid.

EXPERIMENTAL

Fractionation of Oil

Dry, powdered seed of *Vernonia volkameriaefolia* (25 g) were extracted in a Sohxlet with petroleum ether (40-60 C); the product obtained after removal of the solvent (5 g) was esterified with methanol (10 mL) containing sodium (0.05 g) in dichloromethane (65 mL). Fractionation was achieved by silica gel column chromatography. Elution with petroleum ether afforded fraction I, while succesive elution with 4% diethyl ether and 15% diethyl ether in petroleum ether yielded fractions II and III.

Gas Chromatography (GC)

The gas liquid chromatography (GLC) of fatty acids, derived as their methyl esters, was carried out with a stainless-steel column (2m × 3mm inner dimension) containing 15% DEGS on Chromosorb W using a Perkin Elmer model 154 chromatograph, column temperature 200 C. Gas chromatographic retention times were compared with those of authentic compounds available from earlier studies.

Mass Spectrometry

Mass spectra were recorded on an M5902 instrument, source temperature 200 C and ionization potential 70eV. Samples were introduced by the direct insertion technique. Accurate mass measurements were made on an MS30 mass spectrometer coupled to a DS50 SM data system using perfluorokerosene as the internal standard.

Infrared Spectroscopy (IR)

IR analysis was performed, as liquid films, with a Perkin Elmer 621 spectrophotometer.

Nuclear Magnetic Resonance (NMR)

Proton NMR spectra were obtained using a Varian A-60 instrument.

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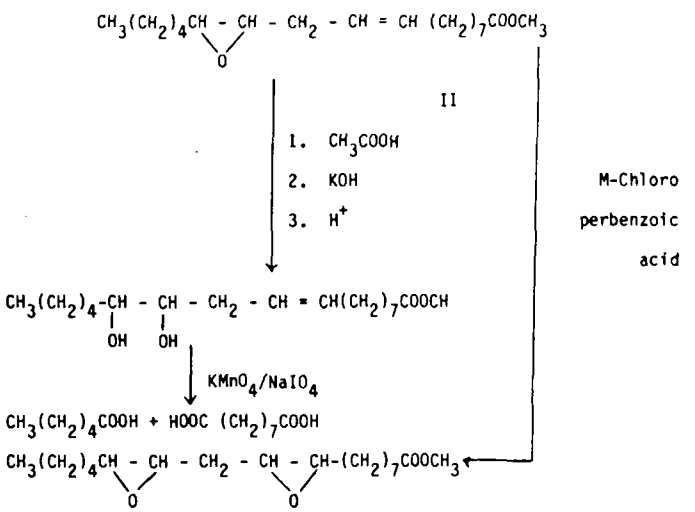

SCHEME 1

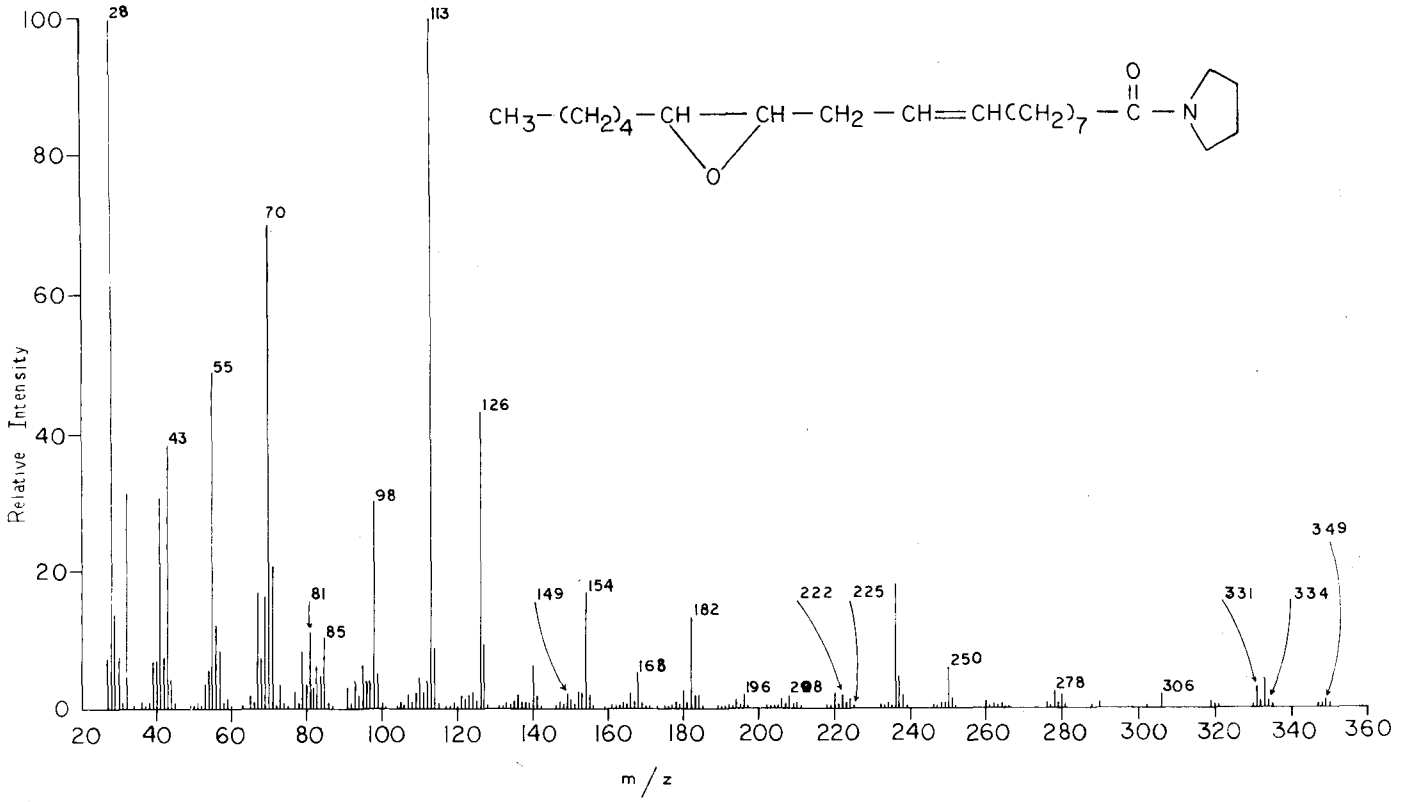

FIG. 1. Mass spectra of pyrrolidide derivative of fraction II.

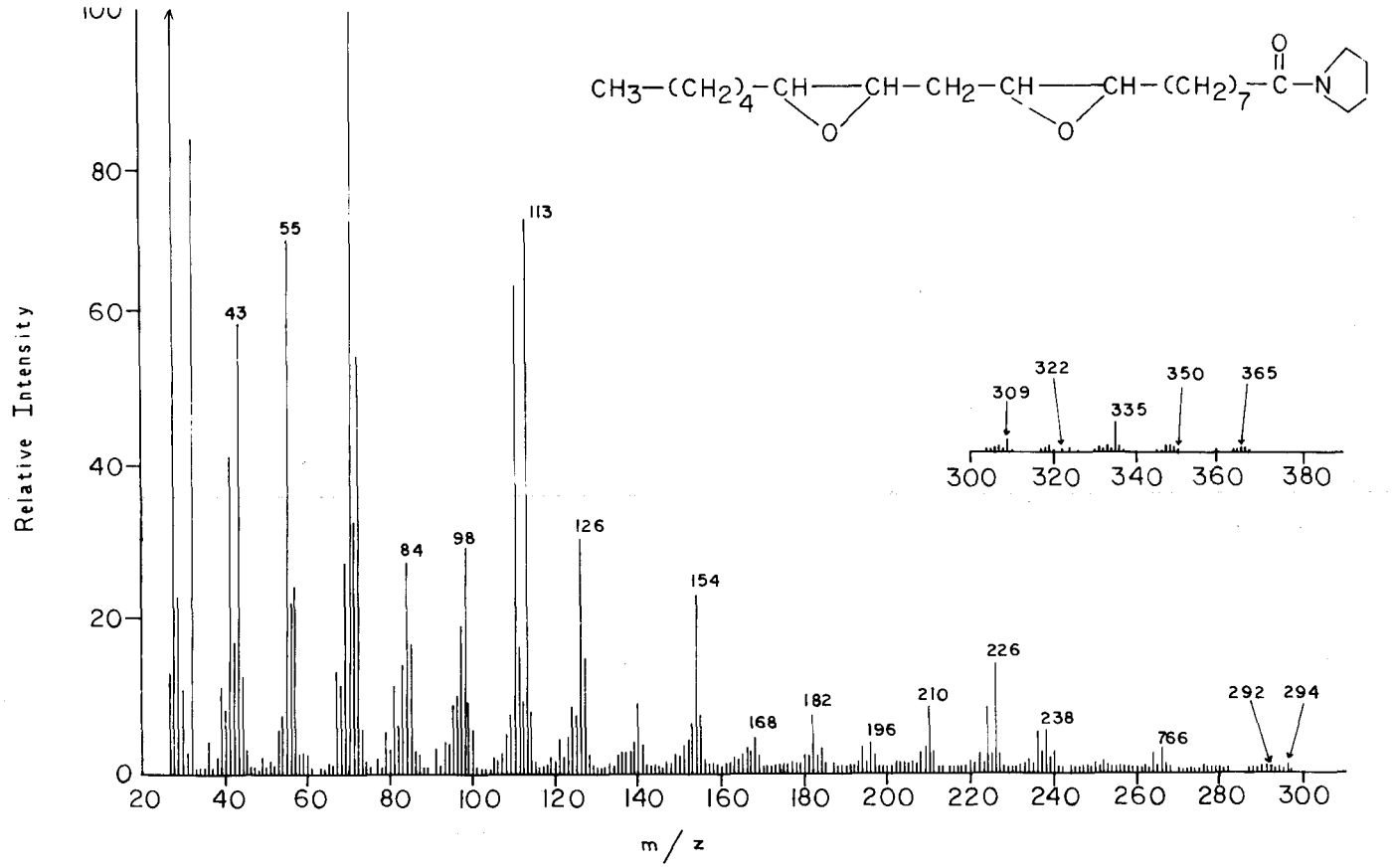

FIG. 2. Mass spectra of pyrrolidide derivative of fraction II diepoxide.

Thin Layer Chromatography (TLC)

Analytical TLC was performed on silica gel plates using petroleum ether/diethyl ether (7:3 v/v) as the developing system. Argentation TLC was performed using silica gel plates impregnated with 20% silver nitrate. The developing system was petroleum ether/diethyl ether (65/35, v/v).

RESULTS AND DISCUSSION

TLC, using both silica gel and silver nitrate impregnated silica gel, of the crude extracted oil of *Vernonia volkameriaefolia* and its methyl ester derivatives showed many components, including oxygenated and unsaturated compounds. HBr titration (3) of the oil at 3 C and 55 C indicated 63.5% epoxyoleic acid. The IR of the crude mixture of the methyl ethers showed a weak band at 3400 cm^{-1} (hydroxyl group) and sharp bands at 840 cm^{-1} and 820 cm^{-1} characteristic of an epoxy group. The crude oil did not give a positive reaction in the Halphen test for cyclopropyl fatty acids (4) but did give a positive picric acid test, characteristic of epoxy compounds (5). Esterification of the crude oil and subsequent fractionation by column chromatography afforded 3 fractions, which were classified by TLC, color reactions and IR as nonoxygenated compounds (I), epoxygenated material (II) and hydroxy-containing compounds (III). Fraction II, which was homogeneous by TLC, gave an IR possessing bands at 3010 cm^{-1} (*cis* double bond (6)), 1740 cm^{-1} and 1170 cm^{-1} (ester) and at 840 cm^{-1} and 820 cm^{-1} (*cis* epoxide (7)). Microanalysis gave C, 75.2; H, 11.4%, calculated for $C_{19}H_{34}O_3$, C, 75.5, H, 11.0%. The 60 MHz NMR spectrum contained a 2-proton multiplet (δ 5.42), characteristic of a *cis* double bond. The spectrum was very similar to that of methyl *cis*-12,13-epoxy-*cis*-9, 10-octadecenoate isolated from *Vernonia anthelmintica*.

Epoxidation of II with m-chloroperbenzoic acid and methylene dichloride (8) afforded a product (found C, 69.1; H, 11.1% calculated for $C_{19}H_{34}O_4$; C, 69.9; H, 10.4%) that exibited NMR and IR characteristic of a *cis, cis*-diepoxyalkanoate. In particular, the IR spectrum lacked the band at 3010 cm present in fraction II.

Acetolysis of fraction II, by refluxing with glacial acetic acid, and saponification to yield an unsaturated diol and subsequent oxidative cleavage with permanganate/periodate reagent (9) afforded 2 products that were esterified with diazomethane and analyzed by GC and TLC. Such analysis, together with a comparison with authentic standards, showed the 2 cleavage products to be methyl hexanoate and dimethyl azeleate. Thus chromatographic, spectroscopic and chemical data are consistent with fraction II being methyl vernoleate, methyl *cis*-12,13-epoxy-*cis*-9,10-octadecenoate, (Scheme 1). Additional confirmation was obtained by a detailed analysis of the mass spectra of II, its epoxide and their pyrrolidide derivatives (10), the latter having been shown to yield particularly useful structural information (11-13).

While the mass spectra of the 2 methyl esters gave weak molecular ions and ions at m/z 99 ($C_6H_{11}O$) and 113 ($C_7H_{13}O$) that were suggestive of an oxygen being of an oxygen being attached to C_{12}-C_{13}, the pyrrolidides were much more useful (Fig. 1 and 2). The normal mode of pyrrolidide fragmentation yields the homologous sequence m/z 113, 126, 140, 154, 168, 182 and 196 characteristic of the $-(CH_2)_7CO_2H$ moiety. The sequence was interrupted at m/z 208, indicating a double bond in the C9-10 position and, after the resumption of normal sequence ions (m/z 222, 236, and 250), a second interruption occurred at m/z 278, placing the epoxide function at C12-13 (10,12).

Similar analysis of the mass spectrum of the diepoxide (Fig. 2) locates the 2 epoxide rings at C 9-10 and C 12-13. Such analysis, however, can offer no information as to the stereochemistry of the unsaturation.

TLC of fraction III showed 2 components with very similar mobilities. Preliminary analysis and comparison with standard compounds suggested that these were the methyl esters of 12, 13-dihydroxyoleic acid and 9,10-dihydroxystearic acid. However, further work is needed to unambiguously assign these structures.

On the basis of the above work and the GLC analysis of fraction I, the fatty acid composition of *Vernonia volkameriaefolia* seed oil may be stated as 16:0 (3.4%); 18:0 (1.3%); 18:1 (3.9%); 18:2 (22.5%); 18.3 (4.1%); epoxy acid (63.5%) and dihydroxy acid (1.5%).

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