Acid-Catalyzed Alcoholysis of *Vernonia galamensis* Oil

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We have demonstrated the potential of *Vernonia galamensis* seed oil as a source of hydroxy alkoxy fatty esters. Reaction of the oil with various alcohols (methanol, ethanol, 1-propanol and 2-propanol), under acidic conditions, resulted in transesterification as well as epoxy ring opening in all cases. The major products, the hydroxy alkoxy fatty esters, constituted approximately 80% of the product mixtures, of which the 12-hydroxy-13-alkoxy isomers were the major constituents. These derivatives were isolated by solvent extraction and/or column chromatography to afford 78–80% of pure isomers. Alcoholysis with butanol resulted in a poor yield of the hydroxy butoxy esters. A discussion of the isolation and mass-spectrometric characterization of these new esters is provided.

KEY WORDS: Acetylation, acid-catalyzed alcoholysis, hydroxy alkoxy, esters, infrared spectroscopy, isomeric mixture, mass spectrometry, proton NMR, selective epoxy opening, transesterification, *Vernonia galamensis*.

Vernonia galamensis is an annual herb native to semi-arid regions of Africa. Commercial development of the plant is now underway in Zimbabwe, Kenya, Ethiopa and Costa Rica (1,2). Additionally, screening experiments are currently being undertaken by the United States Department of Agriculture (USDA)/Agricultural Research Service (ARS) in Arizona. The seed of the herb contains about 40% oil, of which the vernolic (*cis*-12,13-epoxy-*cis*-9-octadecenenoic) acid content is 72–80%; palmitic acid, 2.7–3.3%; stearic acid, 2.7–3.9%; oleic acid, 3.6–5.6% and linoleic acid, 12.6–14.0% (3,4). Thus with the high vernolic acid content, the oil constitutes a viable synthetic starting material for hydroxy alkoxy acids and esters.

Previous studies in our laboratory have noted that base-catalyzed transesterification of vernonia oil afforded the epoxy ester, and saponification with potassium hydroxide did not cleave the epoxy ring (4). On the other hand, in earlier work by Kleiman and co-workers (5,6), directed at developing an analytical method for the gas chromatographic analysis of vegetable oils containing oxygenated fatty acids, it was noted that acid-catalyzed methanolysis resulted in epoxy ring opening to afford the hydroxy methoxy esters. In their investigations, oils from *Vernonia anthelmintica* and *Euphorbia lagascae* were used, and they reported that the acid-catalyzed reaction generated two hydroxy alkoxy isomers, which were silylated and subsequently characterized by gas chromatography/mass spectrometry (GC/MS) as 12-hydroxy-13-alkoxy and 13-hydroxy-12-alkoxy isomers. The isomeric ratio, however, was not determined due to co-elution under the gas chromatographic conditions. Furthermore, because their work was based on developing a micro-analytical technique, no effort was made to ascertain a distinct isomeric ratio for the derivatives. One would, however, expect that due to the greater steric hindrance on carbon-12, any nucleophilic attack on the epoxy ring would favor the carbon-13 position.

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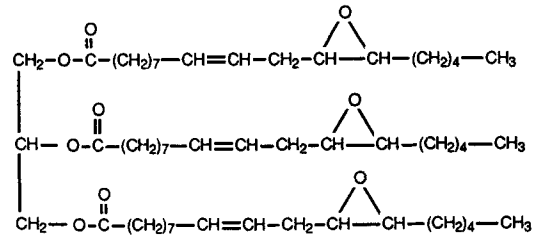
As a part of our study on the synthetic applications of vernonia oil, we have investigated the effect of mineral acid catalysis on the alcoholysis of the oil. Methanol, ethanol, 1-propanol and 2-propanol gave quantitative yields of the corresponding hydroxy alkoxy esters. The reaction with 1-butanol, however, gave a poor yield of the hydroxy butoxy esters in addition to a dark viscous product mixture.

A discussion of the isolation and GC/MS analysis of the hydroxy alkoxy esters is given below. (Scheme 1: trivernolin (major triacylglycerol in *Vernonia galamensis* oil).



SCHEME 1

EXPERIMENTAL PROCEDURES

Crude vernonia oil obtained by mechanical pressing of enzyme-deactivated seed was used in the study. Reactions and products were monitored with a Perkin-Elmer 983G Infrared Spectrophotometer, (Norwalk, CT) and a Finnigan gas chromatograph (GC, model 9611) equipped with a splitless injector and interfaced with a Finnigan MAT 4500 automated mass spectrometer with a SUPERINCOS data system (Finnigan Corp., Sunnyvale, CA). The interface oven and transfer line were maintained at 300°C, ionizer setting at 140°C, electron energy at 70°V and injector temperature at 250°C. The mass spectrometer (MS) was operated in the electron impact (EI) mode with emission current 0.39 mA, and electron multiplier 1500 V. High-resolution capillary gas chromatography was obtained with a Supelco fused silica SPB-1 column (30 m, 0.32 mm i.d., 0.25 μm film) (Bellefonte, PA), with oven temperature programmed from 50°C to 300°C and helium as carrier gas at a head pressure of 10 psi. Activated basic alumina (standard grade, ~150 mesh, 58 Å) was purchased from Aldrich Chemical Company Inc. (Milwaukee, WI) and used in the separations of fatty ester derivatives. Generalized synthetic procedures for the reactions are given below, with the provided data representing average values from several experiments.

Alcoholysis of vernonia oil. For each of the alcohols (methanol, ethanol, 1-propanol, 2-propanol and 1-butanol), a 250-mL round-bottom flask equipped with a reflux condenser and a magnetic stir bar was charged with *ca.* 5 g of vernonia oil (.005 moles, based on a molecular weight of 926), 25 mL of the alcohol and 0.2 mL (0.8% vol/vol) of concentrated sulfuric acid. The mixtures were maintained at their refluxing temperatures and monitored for completion of the reaction by gas-chromatographic analysis of aliquots with squalane as an internal standard.

The ratio of the hydroxy alkoxy products to squalane was plotted at intervals, and reaction completion was assumed when a constant ratio was reached.

Isolation of methyl 12(13)-hydroxy-13(12)-methoxy-9-octadecenoate. To a 500-mL separatory funnel was added the reaction mixture from above, along with 50 mL of dichloromethane and 100 mL of water. The aqueous layer was removed, and the organic layer was transferred to a 250-mL beaker and dried with anhydrous sodium sulfate, and the solvent was stripped off to afford 5.3 g of light-brown oil. The resulting crude oil was dissolved in 100 mL of hexane, then transferred to a 500-mL separatory funnel and subsequently washed 10 times with 50-mL portions of methanol/water mixture (4:1). The aqueous portions were combined and the solvent was removed in a rotavapor to afford 3.4 g (83% of theoretical yield) of a slightly colored oil that contained only the hydroxy alkoxy isomers.

Isolation of the other hydroxy alkoxy esters. The solutions from the other alcoholysis reactions were solvent-extracted in a fashion similar to the methanolysis reaction to afford quantitative yields of crude oily product mixtures, which, were then transferred to a glass column (24 cm, i.d. 2.5 cm) that was packed with 90 g of basic alumina. Hexane was used to elute the nonhydroxy esters, after which the column was rinsed with methanol to elute the hydroxy esters. The methanol fraction was then stripped to afford light golden oils (*ca.* 80% of theoretical yield).

Acetylation of methyl 12(13)-hydroxy-13(12)-methoxy-9-octadecenoate. In a 250-mL round-bottom flask equipped with a magnetic stir bar, 30 mL (0.318 mol) of acetic anhydride was added to about 5 g (0.015 mol) of the pure isomeric hydroxy methoxy esters. The mixture was refluxed for 1 h, after which water was added and refluxed for another 30 min. The reaction mixture was allowed to cool to room temperature, then transferred to a separatory funnel, and approximately 50 mL of dichloromethane was added. The organic layer was subsequently washed five times with aqueous sodium bicarbonate to remove excess acetic acid, dried with anhydrous sodium sulfate and stripped to afford *ca.* 5.6 g (97% of theoretical yield) of the acetylated esters.

A 5.0-g sample of the acetylated esters was transferred onto a column packed with 85 g basic alumina, and then eluted with hexane to afford 2.2 g of the colorless methyl 12-acetoxy 13-methoxy-9-octadecenoate (b.p. 210°C), the rest of the 12-acetoxy 13-methoxy compound eluting with the 13-acetoxy 12-methoxy compound.

TABLE 1

Reaction Time (h) for Acid-Catalyzed Alcoholysis

Alcohol	Time (h)
Methanol	4
Ethanol	48
1-Propanol	72
2-Propanol	264
1-Butanol[a]	—

[a]Butanolysis reaction was discontinued after 144 h due to the formation of a dark viscous mixture.

RESULTS AND DISCUSSION

The composition of the product mixtures reflected the content of the starting vernonia oil. Infrared spectra of the crude product mixtures were similar and consistent with anticipated structures, showing no epoxy absorption (823 cm^{-1}, 846 cm^{-1}) and a strong hydroxy absorption at 3400 cm^{-1}. For the hydroxy methoxy methyl esters, the ^1H-nuclear magnetic resonance (NMR) data showed the methyl protons of the ester functionality at 4.75 ppm and the methyl protons of the methoxy group at 4.41 ppm.

GC was used to follow the reaction, with squalane as an internal standard. Reaction times significantly increased with the carbon number of the alcohol (Table 1). Introduction of branching in the alcohol (2-propanol *vs.* 1-propanol) dramatically increased the reaction time. GC/MS analysis showed that the derivatives were positional hydroxy alkoxy isomers with close retention times (Fig. 1). To further confirm the isomeric ratio of the hy-

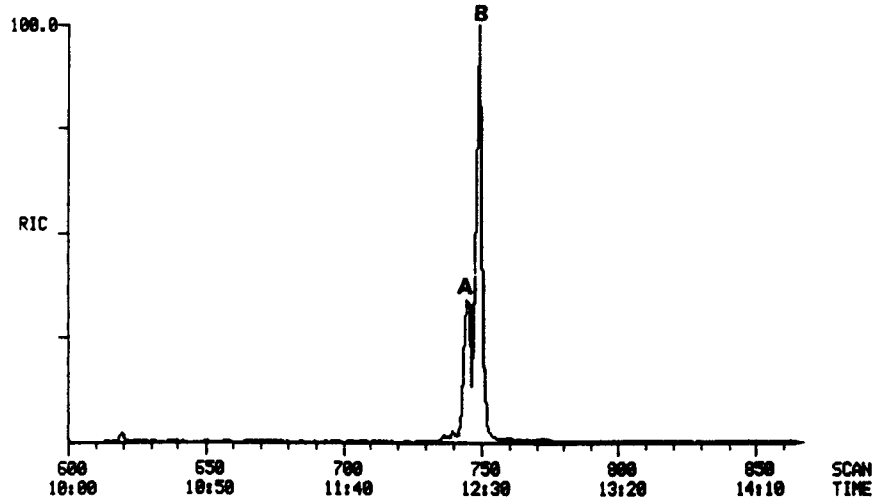


FIG. 1. Reconstituted ion chromatogram of the isolated hydroxymethoxy methyl esters. Peak A = methyl 13-hydroxy-12-methoxy-9-octadecenoate. Peak B = methyl 12-hydroxy-13-methoxy-9-octadecenoate.

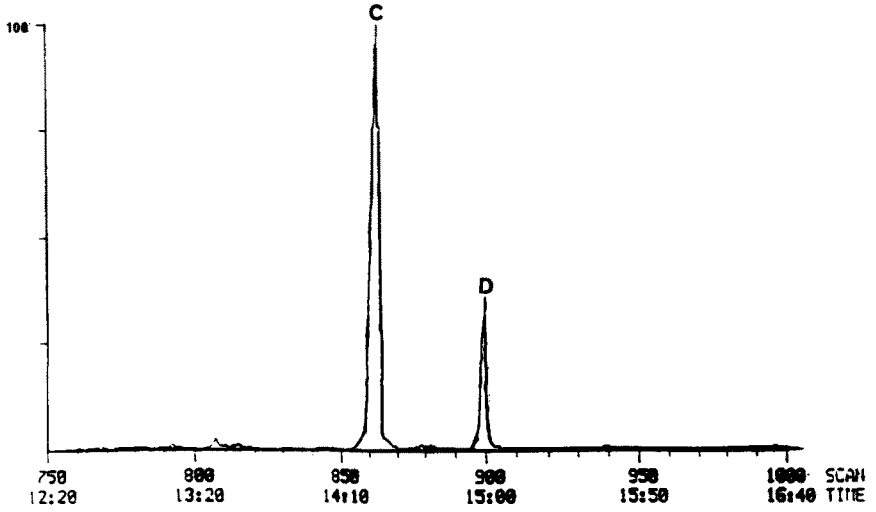


FIG. 2. Reconstituted ion chromatogram of the acetylated hydroxymethoxy methyl esters. Peak C = methyl 12-acetoxy-13-methoxy-9-octadecenoate; Peak D = methyl 13-acetoxy-12-methoxy-9-octadecenoate.

droxy methoxy methyl esters, they were derivatized to the acetates to produce a better resolution on the GC column (Fig. 2).

Table 2 gives the mass-spectral fragmentation ions for some of the diagnostic species in the various derivatives. The major product from the methanolysis reaction (peak B, Fig. 1) gave a mass spectrum (Fig. 3) that exhibited major peaks at *m/z* 55 (65), 115 (100), 145 (44), 166 (33), 195 (56), 227 (43), 311 (2), 342 (<1). A weak molecular ion was at *m/z* 342, and the peak at *m/z* 311 suggested the loss of a methoxy group from the molecular ion. Cleavage between C-12 and C-13 accounted for the diagnostic ion at *m/z* 227 (hydroxy functionality is on C-12) and a fragment ion at *m/z* 115 (methoxy group on C-13).

For the minor component (peak A, Fig. 1) the mass-spectral data exhibited a similar fragmentation pattern. Diagnostic ions in this spectrum included *m/z* 342, (<1), 311 (2), 241 (12), 210 (12), and 145 (100). A weak molecular ion is seen at *m/z* 342, with the loss of a methoxy group being indicated by a peak at *m/z* 311. An ion at *m/z* 241 suggested cleavage between C-12 and C-13 with the methoxy group on C-12. A peak at *m/z* 210 indicated loss of a methoxy group from *m/z* 241, and the fragment at *m/z* 145 was derived from a cleavage between C-11 and C-12. The fragmentation pattern exhibited by the above methyl esters were similar to those reported by Spencer *et al.* (7). Also, the fragmentation patterns of the other hydroxy alkoxy esters were similar to those of the trimethylsiloxy derivatives reported by Kleiman and Spencer (6).

Acetylation of the hydroxy methoxy methyl esters was prompted by two considerations: (i) an effort to obtain a better resolution of the isomers on the gas-chromatographic column, and (ii) the need to ascertain that the fragmentation pattern of each isomer was not complicated by cross-over ions. The methyl 12-acetoxy 13-methoxy-9-octadecenoate afforded comparable fragmentation patterns to its hydroxy methoxy methyl ester. We concluded that the gas-chromatographic resolution of the hydroxy methoxy methyl esters was sufficient for determining isomeric ratios and or structural elucidation.

TABLE 2

Mass-Spectrometric Diagnostic Ions for Hydroxy Alkoxy Esters

	M^+	M^+-OR	C-11–C-12 cleavage	C-12–C-13[a] cleavage
Methyl esters				
12-Hydroxy	342	311	145	227/115
13-Hydroxy	342	311	145	241/NO
Ethyl esters				
12-Hydroxy	370	325	159	214/129
13-Hydroxy	370	325	159	269/101
n-Propyl esters				
12-Hydroxy	NO[b]	339	173	255/143
13-Hydroxy	NO	339	173	297/NO
2-Propyl esters				
12-hydroxy	NO	339	173	255/143
13-Hydroxy	NO	339	173	297/101
n-Butyl esters				
12-Hydroxy	NO	353	187	269/157
13-Hydroxy	NO	353	187	325/NO

[a]Ions on the left correspond to fragments on the ester side of the molelcule.
[b]NO = not observed.

The butanolysis reaction, after 144 h, resulted in a dark viscous reaction mixture which contained little of the hydroxy butoxy esters. The reaction was discontinued at this time, and transesterification of the mixture did not afford any new esters, which indicated that there was no triglyceride left in the reaction mixture. The viscous nature of the isolated crude product suggested the possibility of polymerization, an outcome that might have been caused by the high reaction temperature (118°C). There was no attempt to further characterize the crude butoxy reaction products.

A consistent isomeric ratio of 4:1 (in favor of the 12-hydroxy alkoxy ester) for the different isomeric mixtures suggests a definite preference of the alcohol to attack

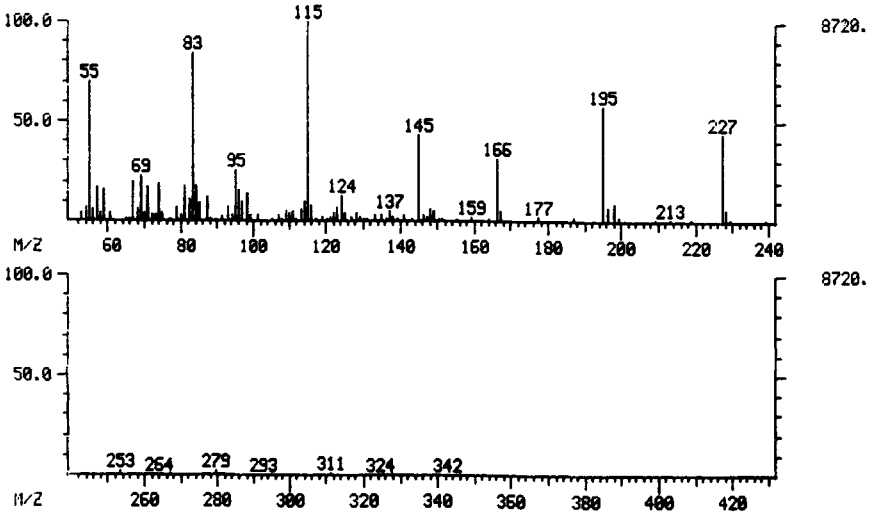


FIG. 3. Mass spectrum of methyl 12-hydroxy-13-methoxy-9-octadecenoate. *m/z* 342 (M^+), 227 [M^+-$CH_3(CH_2)_4CH(OCH_3)$], 145 [$CH_3(CH_2)_4CH(OCH_3)$-$CH(OH)$], 115 [$CH_3(CH_2)_4CH(OCH_3)$]

the epoxy functionality from the C-13 position, thereby placing the hydroxy group on C-12, and as a result of this stereoselective ring opening, it is difficult to justify the formation of *threo* and *erythro* isomers *via* an intermediate carbocation. However, a preferential attack may be due to the greater steric hinderance on C-12, and as such, an S_N2 type mechanism is believed to be operating. Though S_N1 mechanisms are more common in acid catalysis of epoxides, there are known examples of epoxy openings in which an S_N2 type mechanism has been suggested (8,9). In such cases, the carbon-oxygen bond in the epoxide ring is weak, resulting in partial positive charges on both the carbon and the oxygen. Thus, if the substitution on the epoxy ring is unsymmetrical, then alcohol attack would favor the less sterically hindered carbon. Consequently, one would expect that any acid-catalyzed nucleophilic attack on the epoxy functionality in vernonia oil or vernolic acid should preferentially place a hydroxy group on C-12.

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