METHOD

4-Hydroxy-3-methoxycinnamate Esters of Milkweed Oil: Synthesis and Characterization

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ABSTRACT: The common milkweed (Asclepias syriaca L.) is a new industrial crop. Its seed oil (TAG) is highly polyunsaturated. In the search for novel applications for milkweed seed oil, the olefinic groups in the TAG were oxidized to polyhydroxy TAG via epoxidation and subsequent epoxy ring-opening reactions. These polyhydroxy TAG exhibit unique industrially desirable emulsion properties in water. Esterification of the secondary polyhydroxy functionalities of the TAG derivatives of the oil with trans-4-hydroxy-3-methoxycinnamic acid (ferulic acid) has resulted in the development of novel cinnamate esters of milkweed oil. These cinnamates are also obtainable via direct ring-opening of the epoxy TAG intermediate with ferulic acid. Among the interesting characteristics of the ester derivatives is their UV radiation-absorbing property.


For a new industrial crop to achieve economic success, its components must have unusual properties that set it apart for new markets. Such markets are usually small-volume, value-added niches. The higher-value products give incentives to the farmer to undertake the initial capital investment necessary to cultivate the new crop. The situation for the common milkweed (Asclepias syriaca L.) would have presented a double impediment to commercialization because this species is viewed as a nuisance by most farmers who have encountered it in their crop fields or in the range. But in spite of its image, milkweed in recent years has again become a new industrial crop because of the market demand for its floss (fiber) in hypoallergenic pillows and comforters. Interestingly, the floss is the same component of the seed that made milkweed a strategic material during World War II (1). But it was the application of carbocation chemistry that enabled estolides to be prepared on a pilot scale from unsaturated carboxylic acids that have no hydroxyl functional groups (9). Mechanistically, unlike the traditional condensation reaction between an alcohol moiety of an acyl chain and a free carboxylic acid to form estolides, a larger-scale estolide reaction depends on the initial generation of an electrophile at one end of a C=C bond as a result of protonation of the double bond by a Brønsted acid catalyst. The resulting electrophile then accepts an electron pair from the carboxyl OH of a neighboring carboxylic acid moiety thus forming the estolide. Overall the reaction is an addition across a C=C bond by the –OH moiety of a carboxylic acid. These reactions have only been successful with monounsaturated carboxylic acids (10–12). Compton and coworkers (13) used lipase-catalyzed transesterification of soybean oil with ferulic acid ethyl ester to produce UV-absorbing glycerides. The present study reports the novel synthesis of a UV-absorbing estolide by condensation of milkweed polyhydroxy TAG with trans-4-hydroxy-3-methoxycinnamic acid (ferulic acid) to give the cinnamate ester of the intact TAG. This ester is also accessible by direct ring-opening of milkweed epoxy TAG with 4-hydroxy-3-methoxycinnamic acid in the presence of an acid catalyst.

MATERIALS AND METHODS

Materials. Crude, cold-pressed milkweed oil was obtained from Natural Fibers Corporation (Ogallala, NE). Activated acid clay (Bentonite) was obtained from Harshaw/Filtrol Clay Products Division, (Jackson, MS). Sample centrifugation was performed using a Beckman Coulter centrifuge, model J2-HS (Beckman Coulter, Inc., Fullerton, CA). Formic acid, 96% was

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Abbreviations: Ferulic acid, trans-4-hydroxy-3-methoxycinnamic acid; VLC, vacuum LC.

TABLE 1

<table>
<thead>
<tr>
<th>FA Composition of Milkweed (Asclepias syriaca) Oil</th>
<th>% Content</th>
<th>% Content</th>
</tr>
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<tbody>
<tr>
<td>Acid type</td>
<td></td>
<td>Acid type</td>
</tr>
<tr>
<td>Oleic 31.0 (Δ9, Δ11)</td>
<td>Palmitoleic 9.6 (Δ9, Δ11)</td>
<td></td>
</tr>
<tr>
<td>Linoleic 50.5</td>
<td>Palmitic 5.7</td>
<td></td>
</tr>
<tr>
<td>Linolenic 1.2</td>
<td>Stearic 2.5</td>
<td></td>
</tr>
</tbody>
</table>

*From Reference 2.
obtained from Fisher Scientific (Chicago, IL); hydrogen peroxide, 50% in water, and anhydrous ZnCl₂ powder were from Aldrich Chemical Company (St. Louis, MO). 1H and 13C NMR spectra were obtained on a Bruker ARX-400 with a 5-mm dual proton/carbon probe (Bruker Spectrospin, Billerica, MA); the internal standard used was tetramethylsilane. Specific rotation \(\left[\alpha\right]_{D}^{20}\) values were measured on a PerkinElmer Polarimeter model 341 (PerkinElmer, Norwalk, CT).

Viscosity measurements. Viscosity measurements were determined in a Temp-Trol viscosity bath (Precision Scientific, Chicago, IL) using Cannon-Fenske viscometers for transparent liquids (Cannon Instrument Company, State College, PA) in accordance with AOCs Official Method Tq 1a-64 (14). The size of the Cannon-Fenske viscometer used was number 400 (378E) or 300. The cleaned dry tube was loaded at room temperature with the sample oil and placed in its holder in the constant-temperature bath. The sample was allowed to equilibrate for 10 min at 40°C or 15 min at 100°C before the sample was suctioned into the lower bulb until the meniscus just overshot the mark above the lower bulb. The suction was removed and the meniscus adjusted to the mark. The sample was allowed to flow at the same time the stop clock was started. The duration (in seconds) it took the meniscus to reach the mark below the bulb multiplied by the tube constant gave the viscosity of the fluid. The measurement was replicated for reproducibility.

FTIR spectrometry. Test samples of the reaction products were pressed between two NaCl discs (25 × 5 mm) to give thin transparent oil films for analysis by FTIR spectrometry. Spectra were measured on a Bomem Arid Zone FTIR spectrometer (Bomem MB-Series; Bomem, Quebec, Canada) equipped with a deuterated triglycine sulfate detector. Absorbance spectra were acquired at 4 cm⁻¹ resolution and signal-averaged over 32 scans. Interferograms were Fourier-transformed using cosine apodization for optimal linear response. Spectra were baseline corrected and normalized to the methylene peak at 2927 cm⁻¹.

Methods. (i) Synthesis of epoxy TAG. Refined milkweed oil (186.8 g, 212.1 mmol, iodine value (IV) = 111.4) was placed in a 500-mL three-necked jacketed flask equipped with a mechanical stirrer and heated to 40.5°C. Formic acid (96%, 12.0 g, 1.22 mol) was added to facilitate separation of the phases. After removal of water was achieved, followed with deionized water. Ethanol was added to facilitate separation of the phases. After removal of the aqueous layer, the product was concentrated in vacuo to 70°C to yield 711.6 g (92.1% of the polyhydroxyl TAG with an IV = 14 compared with an IV of 114 in the starting milkweed oil. The measured kinematic viscosities were: 2332.5 cSt at 40°C and 75.53 cSt at 100°C, that is, a viscosity index of 37.6 centistokes°C⁻¹. Specific rotation \(\left[\alpha\right]_{D}^{20}\) = +0.37°. FTIR (film on KBr) cm⁻¹: 3636-3168 ν_m, 2876 s, 2863 m, 2856 m, 2847 s, 1474 s, 1393 ν_m-S, 1240 m, 1173 vs, 1097 s, 881 w, 725 w-m. 13C NMR (CDCl₃) δ: 173.2, 172.8 (–COO–), 68.87 (–HCO–), 56.12 (–CH₂–O–), 57.10 (HCO–epoxy). 57.05 (HCO– epoxy), 56.91 (HCO–O), 56.08 (–COO–), 56.63 (–C–O–C–), 56.55 (COC), 54.52 (COC), 54.09 (–C–O–C–), 34.05 (–CH₂–), 33.90 (–CH₂–), 31.79 (–CH₂–), 31.61 (–CH₂–), 29.63 (–CH₂–), 29.47 (–CH₂–), 29.28 (–CH₂–), 29.23 (–CH₂–), 29.15 (∑CH₂–), 29.12 (∑CH₂–), 28.92 (∑CH₂–), 28.88 (∑CH₂–), 27.83 (∑CH₂–), 27.77 (∑CH₂–), 27.75 (∑CH₂–), 27.16 (∑CH₂–), 26.88 (∑CH₂–), 26.55 (∑CH₂–), 26.52 (∑CH₂–), 26.08 (∑CH₂–), 24.73 (∑CH₂–), 22.51 (∑CH₂–), 13.93 (∑CH₂–).

(ii) Synthesis of polyhydroxy TAG. In a 1-L jacketed flask, as in above setup, was placed reprocessed milkweed oil (648.0 g, 735.7 mmol). The oil was stirred vigorously at 40°C, and formic acid (90.4%, 62.2 g, 1.22 mol) was added in one portion followed by a slow (dropwise) addition of H₂O₂ (50%, 203.0 g, 2.98 mol). At the end of peroxide addition, the temperature was increased to 70°C. After 15 h, the heat source was removed but stirring was continued, allowing the reaction mixture to cool to room temperature; stirring was stopped and the separated aqueous phase was removed. Deionized water (300 mL) was added followed with 6 M HCl (100 mL). The nearly colorless sludge was stirred at 70°C overnight. The cream-colored product was transferred into a separatory funnel using ethyl acetate as diluent. The aqueous layer was discarded and the organic phase was washed sequentially with saturated NaCl solution, then saturated NaHC0₃ until a pH of 7.5 in the wash water was achieved, followed with deionized water. Ethanol was added to facilitate separation of the phases. After removal of the aqueous layer, the product was concentrated in vacuo at 70°C to yield 711.6 g (92.1% of the polyhydroxyl TAG with an IV = 14 compared with an IV of 114 in the starting milkweed oil. The measured kinematic viscosities were: 2332.5 cSt at 40°C and 75.53 cSt at 100°C, that is, a viscosity index of 37.6 centistokes°C⁻¹. Specific rotation \(\left[\alpha\right]_{D}^{20}\) = +0.37°. FTIR (film on KBr) cm⁻¹: 3636-3168 ν_m, 2876 s, 2863 m, 2856 m, 2847 s, 1474 s, 1393 ν_m-S, 1240 m, 1173 vs, 1097 s, 881 w, 725 w-m. 13C NMR (CDCl₃) δ: 173.2, 172.8 (–COO–), 68.87 (–HCO–), 56.12 (–CH₂–O–), 57.10 (HCO–epoxy). 57.05 (HCO– epoxy), 56.91 (HCO–O), 56.08 (–COO–), 56.63 (–C–O–C–), 56.55 (COC), 54.52 (COC), 54.09 (–C–O–C–), 34.05 (–CH₂–), 33.90 (–CH₂–), 31.79 (–CH₂–), 31.61 (–CH₂–), 29.63 (–CH₂–), 29.47 (–CH₂–), 29.28 (–CH₂–), 29.23 (–CH₂–), 29.15 (∑CH₂–), 29.12 (∑CH₂–), 28.92 (∑CH₂–), 28.88 (∑CH₂–), 27.83 (∑CH₂–), 27.77 (∑CH₂–), 27.75 (∑CH₂–), 27.16 (∑CH₂–), 26.88 (∑CH₂–), 26.55 (∑CH₂–), 26.52 (∑CH₂–), 26.08 (∑CH₂–), 24.73 (∑CH₂–), 22.51 (∑CH₂–), 13.93 (∑CH₂–).

(iii) Synthesis of milkweed cinnamate from the polyhydroxy TAG. Milkweed polyhydroxy TAG (34.40 g, 32.74 mmol), glacial acetic acid (150 mL), ferulic acid (45.0 g) and ethyl acetate (250 mL) were placed in a 1-L three-necked round-bottomed flask equipped with a
mechanical stirrer. The contents of the reaction flask were stirred and heated to gentle reflux. Progress of the reaction was monitored by TLC (hexanes/ethyl acetate: 1:1 vol/vol) on precoated silica gel. After 48 h the reaction mixture was allowed to cool to room temperature, diluted with more ethyl acetate, and transferred into a separatory funnel. The solution was washed with deionized water (300 mL x 4) to remove most of the acetic acid. The organic phase was then washed with saturated disodium hydrogen phosphate solution and deionized water until the washings were about pH 7. The red-tinted organic solution was dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuo to give a crude product (64.0 g, 93.1% yield). The crude product was purified by a vacuum LC (VLC) technique on silica gel with hexanes/ethyl acetate (1:1) as the eluting solvent. The yield of the desired fraction, i.e., without free ferulic acid) was 44.50 g (64.7%) based on the hexaferuloyl estolide. 1H NMR values on normal silica gel were 0.36, 0.42, and a major band 0.57, compared with 99% ferulic acid, which gave two spots of $R_f$ 0.18 and 0.21: $[\alpha]_D^{20}$ = +0.12$^\circ$ (0.05, CH$_2$Cl$_2$), UV absorption maxima were at 322.5 and 294.5 nm. FTIR (film on NaCl cm$^{-1}$: 3554 (phenolic OH stretch) b, 3014 (=C–H) m, 2967 (CH$_3$– asym.) s, 2939 (–CH$_2$– asym.) vs, 2876 (CH$_3$ sym.) s, 2863 (–CH$_2$– sym. stretch) vs, 1753 (–OC=O TAG) vs, 1714 (–OC=O feruloyl) m, 1640 (–C=– alkene puckering) m, 1612, 1523 (–C=C– arom. breathing mode) m-s, 1465 (–CH$_3$– deformation) m-s, 1375 (–CH$_2$– umbrella effect) m, 1277 (–COC– ester) m, 1259 (–COC– ester) w, 1185 (–OC–CHO–) m-s, 1042 (C–CH$_2$– O– stretch) w, $^1$H NMR (CDCl$_3$) $\delta$: 7.64 (J = 15.9 Hz, trans H–C=C–H, 2H), 7.08 dd (J = 8.2 Hz, arom. ortho coupling, 2H), 7.05 d (J = 2.0 Hz, arom. meta coupling, 2H), 6.93 d (J = 8.2 Hz, arom. ortho coupling 2H), 6.30 d (J = 15.9 Hz, trans C=C–H coupling, 2H), 4.26 q (J = 14.3 Hz, 7H), 4.19 q (J = 14.3 Hz, 7H), 3.88 s (–OCH$_3$, 9H), 2.31 t (J = 7.5 Hz, 7H), 2.11 m (8H), 1.62 m (8H), 1.33 m (90H), 0.90 t (J = 7.0 Hz, 9H). $^{13}$C NMR (CDCl$_3$) $\delta$: 173.9 (4 overlapping lines C=O), 170.7 (2 lines C=O), 167.4 (C=O), 148.0 (ipso arom.), 146.0 (ipso arom.), 144.7 (arom.), 127.1 (arom.), 123.1, 115.7, 114.8, 109.4 (arom.), 75.12 (–OCH$_3$), 74.85 (–OCH$_3$), 73.05 (–OCH$_3$), 72.45 (–OCH$_3$), 69.22 (–OCH$_3$), 68.79 (–OCH$_3$), 65.46 (–HCO$_2$), 60.43 (–CH$_2$O), 60.25 (–CH$_2$O), 56.00 (–OCH$_2$), 34.97 (–CH$_3$), 34.74 (–CH$_3$), 34.40 (–CH$_3$), 32.00 (–CH$_3$), 31.89 (–CH$_3$), 31.82 (–CH$_3$), 31.72 (–CH$_3$), 31.32 (–CH$_3$), 31.28 (–CH$_3$), 29.76 (–CH$_3$), 29.72 (–CH$_3$), 29.66 (–CH$_3$), 29.58 (–CH$_3$), 29.52 (–CH$_3$), 29.42 (–CH$_3$), 29.33 (–CH$_3$), 29.28 (–CH$_3$), 29.22 (–CH$_3$), 29.13 (–CH$_3$), 29.08 (–CH$_3$), 28.90 (–CH$_3$), 26.50 (–CH$_3$), 26.40 (–CH$_3$), 26.34 (–CH$_3$), 25.62 (–CH$_3$), 25.35 (–CH$_3$), 25.06 (–CH$_3$), 25.03 (–CH$_3$), 24.98 (–CH$_3$), 22.76 (–CH$_3$), 22.72 (CH$_3$), 22.62 (–CH$_3$), 22.55 (–CH$_3$), 21.12 (–CH$_3$), 21.03 (–CH$_3$), 14.43 (–CH$_3$), 14.32 (–CH$_3$), 14.11 (CH$_3$).

**RESULTS AND DISCUSSION**

**Epaxy TAG.** The oxiranes of the common milkweed TAG were prepared as described above (Scheme I) by oxidation of the C–C bonds of the TG using performic acid made in situ.
METHOD

Epoxidation of Milkweed Oil

\[ \text{HCO}_2\text{H} \rightarrow \text{epoxide} \]

**SCHEME 1**

(2,15–18). FTIR spectroscopic analysis of the isolated product showed an absorption band characteristic of the \(-\text{CH-O-CH-}\) stretch of the disubstituted epoxy TAG, that is, a doublet at 824–842 cm\(^{-1}\). The spectrum also showed disappearances of the prominent H–C= stretching mode (3018 cm\(^{-1}\)) as well as the C=C breathing or puckering modes (1654 cm\(^{-1}\)) of the parent olefin. The \(^{13}\text{C}\) NMR distortionless enhancement proton transfer experiment of this intermediate clearly indicated four epoxy units with eight methine carbon resonances at 57.10, 57.05, 56.91, 56.85, 56.63, 56.55, 54.25, and 54.09 ppm. This spectral region was transparent in the starting TAG.

**Polyhydroxy TAG.** The polyhydroxy TAG was synthesized in two ways. In one approach, the isolated oxirane was ring-opened in aqueous mineral acid (HCl) followed by ethyl acetate extraction from the aqueous phase (Scheme 2). The preferred approach was a one-pot reaction starting with the purified milkweed oil oxidation followed in sequence by ring-opening of the intermediate epoxy moieties and final isolation of the polyhydroxy TAG. Although the physical characteristics of this compound have been described elsewhere (2), the main spectroscopic feature observed is its diagnostic broad IR absorption band (3636–3168 cm\(^{-1}\)), which is characteristic of hydrogen-bonded OH stretching frequencies. The derivative of this spectrum shows four well-separated sharp bands corresponding to the symmetric (2863 cm\(^{-1}\)) and the asymmetric (2938 cm\(^{-1}\)) stretching modes of the \(-\text{CH}_2-\); the bands corresponding to the symmetric and asymmetric stretching vibrational modes for the \(-\text{CH}_3-\) moieties are 2876 and 2967 cm\(^{-1}\), respectively. The other major features of this spectrum are the very strong ester carbonyl absorption at 1759 cm\(^{-1}\), the \(-\text{CH}_2-\) deformation at 1473 cm\(^{-1}\), the strong \(-\text{C-C-O-}\) stretch at 1192 cm\(^{-1}\), and the disappearance of the \(-\text{C-O-C-}\) stretch of the epoxy TAG. Confirming the presence of secondary hydroxyl carbons on the alkyl chains as a result of epoxy ring-opening, eight low-field resonance lines (84.60, 83.00, 82.50, 82.00, 80.50, 74.30, 73.82, and 73.20 ppm) corresponding to these carbons were observed in the \(^{13}\text{C}\) NMR spectrum of this compound.

4-Hydroxy-3-methoxycinnamate estolides of Asclepias

**SCHEMES 1 and 2**

TAG. Native milkweed oil is not amenable to direct estolide synthesis because of the polyunsaturated nature of these TAG. A different reaction path was therefore necessary to convert the oil to suitable platforms such as the oxirane and polyhydroxy derivatives. A condensation reaction between the polyhydroxy TAG and ferulic acid catalyzed by concentrated HCl in HOAc and EtOAc gave the estolide in 48 h. A better catalyst for this purpose, however, is the Lewis acid, anhydrous ZnCl₂, which gives the product in about one-third of the above reaction time using toluene as solvent. The analogous reaction with tetrahydroxy jojoba wax catalyzed by ZnCl₂ goes to completion in 10–13 h relative to HCl catalysis (18).

The IR features noticed in the purified estolide are additional bands (3014, 1640, 1612, and 1523 with a weak absorbance at 1714 cm⁻¹). The 3014 cm⁻¹ absorption band corresponds to the = C–H stretch, whereas the breathing modes of the aromatic rings are evident at 1612 and 1523 cm⁻¹. The band at 1640 cm⁻¹ is assignable to the puckering mode of the alkene moiety of the feruloyl species, and the weaker band at 1714 cm⁻¹ is attributable to the conjugated ester –C=O function of the estolide. The estolide is also achievable via a direct epoxy TAG ring-opening reaction with ferulic acid under ZnCl₂ catalysis. The number of cinnamate moieties incorporated in the latter reaction is usually much lower than could be achieved with the polyhydroxy TAG under the same reaction conditions. Using either approach, the estolide obtained shows the same characteristics which in addition to having good lubricity, strongly absorbs both long and short UV wavelengths. The UV spectra show greater absorption intensity for the product derived from the epoxy TAG than that from the polyhydroxy starting material. This is understandable in terms of the extent of reaction obtained when ZnCl₂ is used as catalyst at the reflux temperature of toluene (solvent) compared with the concentrated HCl-catalyzed reaction of the polyhydroxy TAG in refluxing ethyl acetate. This property of strong UV absorbance at very low concentrations makes these TAG derivatives valuable materials that offer effective protection against the increasingly damaging effects of the sun’s UV rays. We have previously shown with trans-4-hydroxy-3-methoxycinnamate derivatives of jojoba oil that the relative intensities of the absorption band maxima between (360 and 260 nm) could be modulated in favor of the shorter-wavelength band (297.5 nm) if desired, while maintaining the same spectral range (18).

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REFERENCES

14. AOCs (1997) Official Methods and Recommended Practices of the AOCs. 5th edn., Method Tq 1a-64, AOCs Press, Champaign.

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