Synthesis of Estolides from Oleic and Saturated Fatty Acids

Steven C. Cermak* and Terry A. Isbell

New Crops Research, NCAUR, ARS, USDA, Peoria, Illinois 61604

ABSTRACT: Oleic acid and various saturated fatty acids, butyric through stearic, were treated with 0.4 equivalents of perchloric acid at either 45 or 55°C to produce complex estolides. Yields varied between 45 and 65% after Kugelrohr distillation. The estolide number (EN), i.e., the average number of fatty acid units added to a base fatty acid, varied as a function of temperature and saturated fatty acid. The shorter-chain saturated fatty acids, i.e., butyric and hexanoic, provided material with higher degrees of oligomerization (EN = 3.31) than stearic acid (EN = 1.36). The individual, saturated fatty acid estolides each have very different characteristics, such as color and type of by-products. The higher-temperature reactions occurred at faster rates at the expense of yield, and lactones were the predominant side products. At 55°C, lactone yields increased, but the δ-γ-lactone ratio decreased; this led to lower estolide yields. The opposite trend was observed for the 45°C reaction. The saturate-capped, oleic estolides were then esterified with 2-ethylhexyl alcohol, and the chemical composition of these new estolides remained consistent throughout the course of the reaction.

Paper no. J9799 in *JAOCS 78,* 557–565 (June 2001).

KEY WORDS: Acid-catalyzed, butyric, decanoic, estolides, hexanoic, lauric, octanoic, oleic acid, palmitic, perchloric acid.

Estolides are a class of esters, based on vegetable oils (1–4), that form when the carboxylic acid functionality of one fatty acid reacts at the site of unsaturation of another fatty acid to form an ester linkage. These linkages are used to help characterize the structure of the estolide since the estolide number (EN) is defined as the average number of fatty acids added to a base fatty acid (Scheme 1, EN = n + 1). The secondary ester linkages of the estolide are more resistant to hydrolysis than those of triglycerides, and the unique structure of the estolide results in materials that have far superior physical properties for certain applications than vegetable and mineral oils (5).

Estolides have been found in nature (6,7) and have been synthesized (1–4,8) in the laboratory. Isbell and Kleiman (9) synthesized estolides from oleic fatty acids and found them to have interesting chemical behavior. The double bond in oleic acid is located at the 9 position which, under the correct conditions of acid concentration, reaction time, and temperature, undergoes estolide formation with minimal migration. The proposed mechanism involves formation of a carbocation that can undergo nucleophilic addition with or without carbocation migration along the length of the chain. If the migration continues to the C4 and C5 position, the fatty acid will cyclize to form lactones, the major side product to estolide formation (Scheme 2) (10–15).

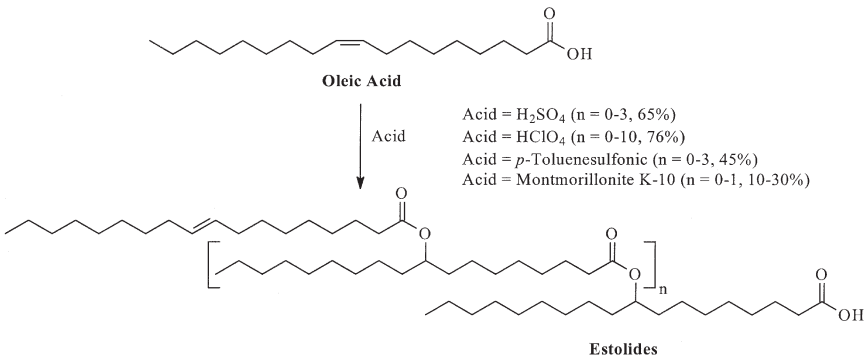
Lactones are formed from an intramolecular cyclization of a fatty acid carboxyl group with a carbocation located at the 4- or 5-position on a fatty acid backbone. Under the correct conditions, Showell *et al.* (13) and Cermak and Isbell (14,15) were able to produce γ- and δ-lactones, respectively. A major side product in the estolide synthesis, lactones are an interesting class of compounds with many possibilities as industrial intermediates. The γ- and δ-lactones display different rates of reaction in the formation of amides from amines (16). This process enables the δ-lactone, the more reactive lactone, to selectively undergo substitution over the γ-lactone to provide high yields of highly desirable 5-hydroxy amides which serve as glucamide-derived biodegradable detergents (17,18).

Estolides and estolide esters from meadowfoam (3,19,20), oleic acid (21), castor oil, or any source of hydroxy fatty acids (22,23) have shown promise as cosmetics, coatings, and biodegradable lubricants. Estolides and estolide esters compare favorably to commercially available industrial products such as petroleum-based hydraulic fluids, soy-based fluids, and petroleum oils, and usually outperform the competition (5). Many estolide ester derivatives have superior biodegradability and lubricating properties compared to petroleum oils (21).

Estolides from oleic acid have been explored intensively in our laboratory (1–4,9,20,21). These estolides were synthesized under various acidic conditions (Scheme 1), with mineral acids providing the best yields and physical properties (2). When one equivalent of $HClO_4$ at 50°C was employed, estolide yields averaged about 76%. The reaction produced a light-colored product that could be used without further distillation for most applications. The use of H_2SO_4 as a catalyst tended to lead to sulfonated by-products that decomposed over time to release sulfonic acid moieties that decreased the pH of the estolide solution, thereby impairing the function of the fluid. The decrease in pH with aging and the dark color of the estolide produced using a H_2SO_4 catalyst are in contrast to the $HClO_4$ reaction which provided a pH-stable estolide with low color.

One of the concerns with oleic estolides is their stability toward oxidation. Oleic estolides show better oxidative stability than both petroleum and vegetable oil-based fluids, but these stabilities can still be improved. For example, Akoh (24) reported that refined soybean oil had an oxidative stability index, OSI (25), of 9.4 h at 50°C. With partial hydrogenation, the OSI increased to 15.3 h at 50°C, an improvement of more than 60%. The same approach was taken with the oleic estolides, which underwent hydrogenation with 2% (w/w) of 10% palladium on activated carbon to obtain completely saturated es-

*To whom correspondence should be addressed at New Crops Research, NCAUR, ARS, USDA, 1815 N. University St., Peoria, IL 61604.
Email: cermaksc@ncaur.usda.gov

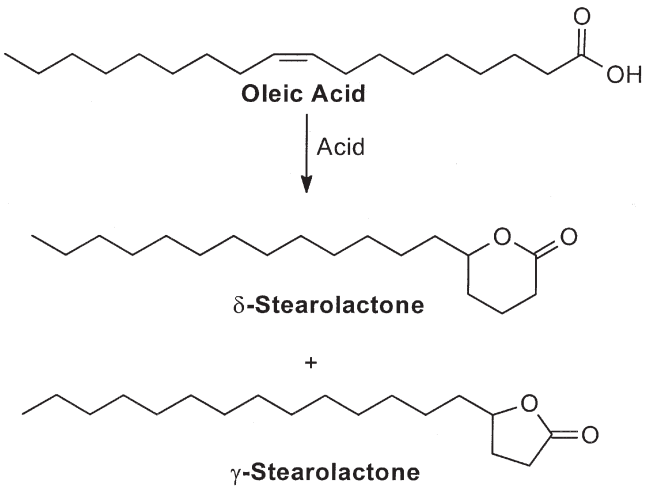


SCHEME 1

tolides (21). The saturated oleic estolides are expected to be much more oxidatively stable than the unsaturated estolides, assuming the same trend displayed by soybean oil holds true. However, pour point was negatively affected (21).

Synthesis of a saturated estolide in one step would increase oxidative stability and reduce production costs and chemical waste. An ideal estolide is envisioned as a completely saturated material that retains good pour point properties. The saturated capping material adds cost to the synthesis of estolides, but improvement in the physical properties may justify the additional expense (26). Certain applications have a high demand for low-temperature properties or high viscosity indices; with proper capping the desired estolide can be synthesized to meet these requirements.

Oleic acid is a desirable starting material because it is readily available from a number of agricultural sources, is relatively inexpensive, and will soon be abundant owing to genetically engineered high-oleic crops. In this paper we report the synthesis of oleic estolides capped with saturated fatty acids, followed by esterification with 2-ethylhexyl alcohol to yield the corresponding esters using standard conditions. We explore alternative reaction methods and alternative fatty acids in order to synthesize saturated, capped, oleic estolides with properties designed for specific industrial applications.



SCHEME 2

EXPERIMENTAL PROCEDURES

Materials. Oleic acid (90%), hexanoic acid (99%), carbon disulfide (spectrophotometric grade), and *N,O*-bis(trimethylsilyl)acetamide (derivatization grade) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Ethyl acetate and hexanes (for extractions), acetone (for high-performance liquid chromatography, HPLC), butyric acid (99+%), octanoic acid (99%), perchloric acid (70%), concentrated sulfuric acid (98%), methanol, 2-ethylhexyl alcohol, and palmitic acid (98%) were purchased from Fisher Scientific Co. (Fairlawn, NJ). Methyl elaidate was purchased from Sigma Chemical Co. (St. Louis, MO). Decanoic acid (99%), myristic acid (97%), lauric acid (97%), and stearic acid (95%) were purchased from Pfaltz & Bauer Inc. (Waterbury, CT). Potassium hydroxide was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ). Filter paper was obtained from Whatman (Clifton, NJ). Acetonitrile and acetic acid (both for HPLC), charcoal, sodium hydrogenphosphate, and sodium dihydrogenphosphate were obtained from EM Science (Gibbstown, NJ). Pyridine was purchased from Mallinckrodt (Paris, KY). The fatty acid methyl ester (FAME) standard mixtures were obtained from Alltech Associates, Inc. (Deerfield, IL). Solvents for chromatography and extraction were HPLC grade, or equivalent, and were used without further purification.

Gas chromatography (GC). This analysis was performed with a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA), equipped with a flame-ionization detector and an autosampler/injector. Analyses were conducted on an SP 2380 30 m × 0.25 mm i.d. column (Supelco, Bellefonte, PA). Saturated C_8–C_{30} FAME provided standards for calculating equivalent chain length (ECL) values, which were used to make fatty acid and lactone assignments.

Two methods for SP 2380 analysis were used to separate the fatty acids. Method A (33.83 min run time) produced a more detailed separation of the shorter fatty acids, including butyric and hexanoic.

Parameters for method A were: column flow 3.3 mL/min with helium head pressure of 15 psi; split ratio 22:1; programmed ramp 120 to 135°C at 10°C/min, 135 to 175°C at 3°C/min, 175 to 265°C at 10°C/min, hold 10 min at 265°C; injector and detector temperatures set at 250°C. Retention

times for eluted peaks (with ECL values in parentheses) were: methyl oleate 10.18 min (18.45), hydroxy methyl oleate 20.25 (26.66), γ-stearolactone 22.20 min (29.69), and δ-stearolactone 22.81 min (30.82).

Parameters for method B were: column flow 3.3 mL/min with helium head pressure of 15 psi; split ratio 22:1; programmed ramp 75 to 165°C at 15°C/min, 165 to 185°C at 7°C/min, 185 to 265°C at 15°C/min, hold 5 min at 265°C; injector and detector temperatures set at 250°C. Retention times for eluted peaks (with ECL values in parentheses) were: methyl oleate 9.73 min (18.36), hydroxy methyl oleate 14.10 min (27.81), γ-stearolactone 15.31 min (30.39), and δ-stearolactone 15.72 min (31.59).

GC–mass spectrometry (GC–MS). GC–MS was performed on a Hewlett-Packard 5890A gas chromatograph with a 30 m × 0.20 mm i.d. SPB1 column (Supelco) and a Hewlett-Packard 5970 mass selective detector. GC conditions: helium head pressure 15 psi at 170°C set for constant flow with varying pressure; split ratio 50:1; injector temperature set at 250°C; transfer line temperature set at 250°C; programmed ramp from 170 to 270°C at 3°C/min. MS conditions: mass range 50 to 550 amu; electron multiplier 200 volts relative.

HPLC. Reversed-phase HPLC analyses were performed on a Thermo Separations Spectra System AS1000 autosampler/injector (Fremont, CA) with a P2000 binary gradient pump from Thermo Separation Products coupled to a Alltech ELSD 500 evaporative light-scattering detector (ELSD). A C-8 reversed-phase analysis used to separate reaction mixtures was carried out with a Dynamax column (250 × 4.5 mm, 5 μm particle size) from Rainin Instrument Co. (Woburn, MA).

Two methods for reversed-phase analysis were used to separate the reaction mixtures. Method A (16 min run time) was used to follow the reaction. It provided information on the overall progress of the reaction. Method B (35 min run time) produced a more detailed separation of the reaction mixture, in particular a separation of estolides, lactones, and hydroxy fatty acids.

Parameters for method A were: flow rate of 1 mL/min; 0 to 4 min 80% acetonitrile/20% acetone; 6 to 10 min 100% acetone; 11 to 16 min 80% acetonitrile/20% acetone. The ELSD drift tube was set at 55°C, with the nebulizer set at 20 psi N_2 providing a flow rate of 2.0 standard liters per minute (SLPM). Retention times for eluted peaks: estolides, 9.8–13.0 min; methyl oleate, 6.3 min; oleic acid, 5.1 min; lactones, 4.8 min; and hydroxy acids, 4.1 min.

Parameters for method B were: flow rate of 1 mL/min; 0 to 2 min 60% acetonitrile/40% acetone; 20 to 25 min 100% acetone; 30 to 35 min 60% acetonitrile/40% acetone. The ELSD drift tube was set at 55°C, with the nebulizer set at 20 psi N_2 providing a flow rate of 2.0 SLPM. Retention times for eluted peaks: estolides, 8.2–25.6 min; methyl oleate, 5.5 min; oleic acid, 4.8 min; γ-lactones, 4.5 min; δ-lactones, 4.1 min; and hydroxy acids, 3.8 min.

Normal-phase HPLC analyses were performed using a Spectra-Physics 8800 ternary pump (San Jose, CA) with a Spectra System AS3000 autosampler/injector from Thermo Separation Products coupled to a Varex ELSD III light-scattering detector (Alltech Associates). A silica normal-phase analysis was carried out with a Dynamax column (250 × 4.6 mm, 8 μm) from Rainin Instrument Co. Components were eluted isocratically from the column with a 80:20 mixture of hexanes/acetone at a flow rate of 1 mL/min with the ELSD drift tube set at 45°C and nebulizer set at 10 psi N_2, flow rate 1.50 SLPM. Normal-phase HPLC was used to separate esterification reaction mixtures. Retention times for eluted peaks: 2-ethylhexyl estolide ester, 2.6–2.8 min; and estolide, 3.5–3.7 min depending on the capping fatty acid.

Acid-catalyzed condensation. These reactions were conducted without solvent in a 500-mL baffled, jacketed reactor with a three-neck reaction kettle cover. The reaction was connected to a recirculating constant-temperature bath maintained at ±0.1°C of the set point. All reactions described were mixed with an overhead stir motor using a glass shaft and a Teflon blade. Two temperatures, 45 or 55°C depending on the reaction, were explored for each set of experiments. Reactions were performed under the general conditions described above while varying the choice of saturated fatty acids as reported in Table 1. In most cases oleic acid (100.0 g, 354.0 mmol) and saturated fatty acids, C_4 through C_{18} (177.0 mmol), were combined and heated to either 45 or 55°C. Once the temperature was reached, perchloric acid (303.4 mmol, 18.3 mL) was added, and the flask was stoppered. Product distribution was monitored by HPLC, GC, and/or GC–MS, as described above. Completed reactions were quenched by the addition of 0.5 M Na_2HPO_4 (212.4 mmol, 424.8 mL) to the reaction vessel. The reactor was disconnected from the circulating bath, and the solution was allowed to cool with stirring for 30 min. The material was transferred to a separatory funnel followed by the addition of 200 mL of a 2:1 ethyl acetate/hexanes solution. The pH of the organic layer was adjusted to 5.3–6.0 with the aid of a pH 5 buffer (NaH_2PO_4, 519 g in 4 L H_2O, 2 × 50 mL) followed by saturated NaCl (2 × 50 mL). The organic layer was dried over sodium sulfate and filtered. All reaction solutions were concentrated *in vacuo* then Kugelrohr-distilled at 160–190°C at 0.1–0.5 mm Hg to remove any lactones, saturated and unsaturated fatty acids.

Synthesis of estolide 2-ethylhexyl ester. The distilled, free acid estolides were combined with a 0.5 M BF_3/2-ethylhexyl alcohol solution [3 × estolide wt, (g) = mLs of 0.5 M BF_3/2-ethylhexyl alcohol]) in a 500-mL round-bottomed flask. The reactions were conducted at 60°C with magnetic stirring and were monitored hourly by normal-phase HPLC as described above. Esterification reactions were run until they were >99% complete, then transferred to a separatory funnel and washed with saturated NaCl (2 × 75 mL). The pH of the organic layer was adjusted to 5.3–6.0 with the aid of pH 5 buffer (NaH_2PO_4, 519 g in 4 L H_2O) (2 × 50 mL). The oil was dried over sodium sulfate and filtered. All reactions were concentrated *in vacuo*, then Kugelrohr-distilled at 100–120°C at 0.1–0.5 mm Hg to remove any excess 2-ethylhexyl alcohol.

Decolorization of 2-ethylhexyl estolide esters. Gardner color of the 2-ethylhexyl estolide esters was measured before

TABLE 1
Acid-Catalyzed Condensation Reaction of Oleic Acid with Varying Saturated Fatty Acids[a]

Estolide	Saturated fatty acid	Temp. (°C)	Percentage estolides[b]	GC estolide number	GC iodine value	% Capped[c]	Gardner color
A	Butyric	45	53.9	3.31	15.3	33.1	8
B	Butyric	55	47.4	2.57	19.3	39.0	11
C	Caproic	45	57.0	3.27	13.9	34.4	9
D	Caproic	55	51.7	3.13	15.5	30.9	11
E	Octanoic	45	58.8	2.89	14.2	42.4	10
F	Octanoic	55	48.9	2.60	17.1	33.7	12
G	Decanoic	45	64.8	2.68	12.0	53.3	18
H	Decanoic	55	56.4	2.50	11.8	57.2	18
I	Lauric	45	63.7	2.20	12.2	58.2	7
J	Lauric	55	60.1	2.11	12.5	59.5	11
K	Myristic	45	64.0	1.81	11.7	64.6	6
L	Myristic	55	54.6	1.81	11.7	64.7	10
M	Palmitic	45	58.5	1.92	10.1	68.4	8
N	Palmitic	55	58.6	1.67	14.0	62.6	10
O	Stearic	45	48.7	1.43	20.9	42.7	11
P	Stearic	55	44.5	1.36	13.7	64.5	11

[a]Reactions were run for 24 h with overhead stirring; reactants were initially present in a 2:1 ratio, oleic/saturated fatty acids in the presence of 0.4 equivalents of perchloric acid.
[b]Yield based on mass of pure estolide obtained *via* distillation.
[c]Ratio of estolide capped with saturated fatty acids as determined by gas chromatography (GC) [SP 2380 column (Supelco, Bellefontaine, PA); 30 m × 0.25 mm i.d.].

and after decolorization on a Lovibond 3-Field Comparator from Tintometer Ltd. (Salisbury, England). To remove color the estolides were placed in a 500-mL Erlenmeyer flask with 2% w/w of activated carbon (Table 2) then stirred with a magnetic stirrer for 4 h at room temperature. Upon completion, hexanes (100 mL) were added and the mixture was filtered through a silica plug. The organic layer was dried over sodium sulfate and gravity filtered. All reactions were concentrated *in vacuo* to remove excess hexanes.

TABLE 2
Esterification with 2-Ethylhexyl Alcohol[a]

Estolide ester[b]	GC estolide number	GC iodine value	Gardner color	Decolorized Gardner color	Gardner color improvement	Acid value (mg/g)
A-2EH	2.84	18.8	11	7	4	0.910
B-2EH	2.95	17.8	13	11	2	1.559
C-2EH	3.46	13.7	11	10	1	0.939
D-2EH	2.69	17.9	13	11	2	0.870
E-2EH	2.96	14.6	11	8	3	1.158
F-2EH	3.07	15.5	14	12	2	1.194
G-2EH	2.69	11.9	18	18	0[c]	1.045
H-2EH	2.30	12.4	18	18	0[c]	1.455
I-2EH	2.16	12.7	12	11	1	0.959
J-2EH	1.92	14.0	15	13	2	0.898
K-2EH	1.98	10.6	11	8	3	0.783
L-2EH	1.77	11.8	14	11	3	1.026
M-2EH	1.35	27.5	18	15	3	0.117
N-2EH	1.13	17.2	17	12	5	1.420
O-2EH	1.09	25.0	12	10	2	0.801
P-2EH	1.13	14.8	14	11	3	0.603

[a]Esterification reactions were run with magnetic stirring and 0.5 M BF_3.
[b]2-Ethylhexyl ester.
[c]There was a visual color improvement, but it was not great enough to affect the Gardner color. For abbreviation see Table 1.

Nuclear magnetic resonance (NMR). ^{1}H and ^{13}C NMR spectra were obtained on a Bruker ARX-400 instrument (Karlsruhe, Germany) with a 5-mm dual proton/carbon probe (400 MHz ^{1}H/100.61 MHz ^{13}C) using $CDCl_3$ as a solvent in all experiments. The assignments of protons are not to the whole number. The NMR spectrum contains a compound that has an average estolide number of 1.90 for the free estolide M and 1.35 for the estolide ester M2-EH, which makes whole number assignment impossible. The data reported for the number of protons in the NMR reflect the actual numbers. The numbers can be multiplied by a factor to obtain whole numbers that correspond to a whole-number estolide number.

^{1}H NMR of free estolide M (Table 1): δ 5.37–5.33 (*m*, 0.5 H, –CH=CH–), 4.86–4.83 (*m*, 1.7 H, –C*H*–OC=O–), 2.32 (*t*, *J* = 7.4 Hz, 2H, –CH_2(C=O)–O–CH–), 2.28–2.23 (*m*, 3.4 H, –CH_2(C=O)–O–CH_2–), 1.96–1.20 (*m*, 74.3 H), and 0.88–0.84 ppm (*m*, 8.3 H, –CH_3). ^{13}C NMR of free estolide M: δ 179.2 (*s*, HO–C=O), 173.7 (*s*, –CH–O–*C*=O), 130.5 (*d*, –*C*H=CH–, very small signal, only a small amount of alkene present), 74.1 (*d*, –*C*H–O–C=O), 34.6 (*t*), 34.0 (*t*), 31.8 (*t*), 29.6 (*t*), 29.6 (*t*), 29.5 (*t*), 29.4 (*t*), 29.3 (*t*), 29.3 (*t*), 29.3 (*t*), 29.2 (*t*), 29.1 (*t*), 29.1 (*t*), 29.0 (*t*), 25.2 (*t*), 25.0 (*t*), 22.6 (*t*), and 14.1 ppm (*q,* –CH_3).

^{1}H NMR of estolide ester M-2EH (Table 2): δ 5.37–5.32 (*m*, 0.4H, –CH=CH–), 4.86–4.81 (*m*, 1.4 H, –C*H*–OC=O–), 3.94 (*d*, *J* = 5.8 Hz, 2.0 H, –OCH_2–CH(CH_2)CH_2–), 2.27–2.22 (*m*, 5.0 H, –CH_2(C=O)–O–CH_2–, –CH_2(C=O)–O–CH–), 1.96–1.22 (*m*, 75.1 H), and 0.87–0.82 ppm (*m*, 12.6 H, –CH_3). ^{13}C NMR of estolide ester M-2EH: δ 174.5 (*s*, C=O), 173.5 (*s*, C=O), 130.0 (*d*, –*C*H=CH–, very small signal, only a small amount of alkene present), 73.8 (*d*, –*C*H–O–C=O), 66.4 (*t*, –O–*C*H_2–CH–), 38.6 (*d*, –CH_2–*C*H(CH_2)–CH_2–), 34.5 (*t*), 34.2

(*t*), 31.8 (*t*), 30.3 (*t*), 29.5 (*t*), 29.3 (*t*), 29.2 (*t*), 29.2 (*t*), 29.1 (*t*), 28.8 (*t*), 25.1 (*t*), 24.9 (*t*), 24.9 (*t*), 23.7 (*t*), 22.9 (*t*), 14.0 (*t*), 24.9 (*q*, $-CH_3$), 13.9 (*q*, $-CH_3$), and 10.8 ppm (*q*, $-CH_3$).

GC analysis of hydroxy fatty acids. Analytical samples for GC were prepared by heating a 10-mg sample of estolide or estolide 2-ethylhexyl ester in 0.5 mL of 0.5 M KOH/MeOH to reflux on a heating block for 60 min in a sealed vial. After cooling to room temperature, 2 mL of 1 M H_2SO_4/MeOH was added to the vial, and the vial was resealed and heated to reflux on a heating block for 30 min. The solution was transferred to a separatory funnel with water (1 mL) and washed with hexanes (2 × 2 mL), dried over sodium sulfate, gravity-filtered, placed in a GC vial with hexanes, sealed, and injected into the GC and/or the GC–MS.

Melting point. Melting points were performed on a Fisher Scientific Co. Fisher-Johns melting point apparatus with a mercury thermometer.

Iodine values (IV) and EN. IV were calculated from the GC results using AOCS Method Cd 1c-85 (25). EN were determined as previously described by GC from the SP 2380 column analysis (9).

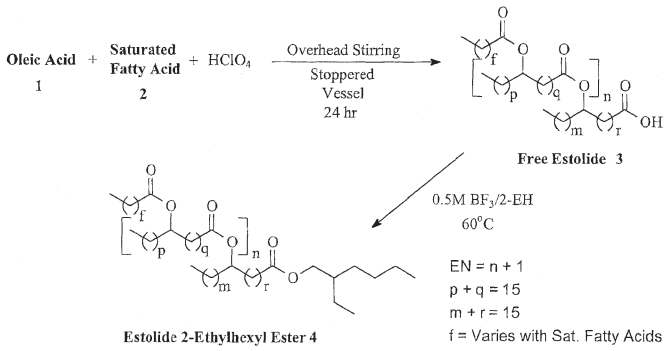
Acid values. The acid values were measured on a 751 GPD Titrino from Metrohm Ltd. (Herisau, Switzerland) by using AOCS Method Te 2a-64 (25), with ethanol substituted for methanol to increase the solubility of the estolide ester during the titration.

Trimethylsilyl (TMS) derivatization of hydroxy fatty esters. Hydroxy fatty acid methyl esters (10 mg) (prepared as described) were dissolved in 0.1 mL of pyridine and 0.05 mL *N,O*-bis(trimethylsilyl)acetamide. This solution was then placed in a sealed vial for 10 min at 100°C. After this time hexanes (1 mL) were added, and the resulting solution was filtered through a silica plug. The filtered sample was placed in a sealed GC vial with hexanes and injected onto the GC and/or GC–MS.

Geometrical isomerization determination of 2-ethylhexyl estolide ester. The degree of geometrical isomerization for the monomers was determined by infrared analysis of the *trans* absorption band at 967 cm^{-1} as described in AOCS Method Cd 14-61 (25) with the modifications published by Lanser and Emken (27). Spectra were obtained from a Bomem Michelson Series FTIR (Québec, Canada) equipped with a Bomem-Grams/32® analytical software program. The monomer (20 ± 0.2 mg) was dissolved in 1.0 mL of CS_2, placed in a 1.0-mm KBr cell, and scanned 32 times with a CS_2 background. Integration of the *trans* absorption band at 967 cm^{-1} and computation of the *trans* isomers with respect to a standard curve developed from methyl elaidate (standards from 5–100% methyl elaidate) provided the percentage of *trans* double bonds present for each monomer fraction.

RESULTS AND DISCUSSION

Table 1 outlines a series of reactions (Scheme 3) that explore the formation of complex estolides at two different temperatures, where a series of different saturated fatty acids, butyric through stearic, are used as the capping material to give the



SCHEME 3

free estolide **3** (Scheme 3). These new estolides have an oleic acid backbone with a terminal fatty acid. Estolides are formed from the carbocationic homo-oligomerization of unsaturated fatty acids (2) resulting from the addition of a fatty acid carboxyl adding across the olefin. This condensation can continue, resulting in oligomeric compounds where the average extent of oligomerization is defined as the EN (Scheme 1) (9). When saturated fatty acids are added to the reaction mixture the oligomerization is terminated upon addition of the saturated fatty acid to the olefin, since the saturate provides no additional unsaturation to further the oligomerization. Consequently, the estolide is stopped at this point from further growth. Thus, the estolide is "capped."

Double-bond migration has been demonstrated under these acidic conditions, with the olefin position eventually spread over the entire fatty acid chain. When the olefin nears the carboxyl we assume that estolide and lactone formation are in equilibrium at the γ- and δ-positions in a classic ring-chain equilibrium typical of other lactones such as caprolactone (28).

Temperature effects on estolide formation. In using oleic acid as a natural source of unsaturated fatty acids, a series of saturated fatty acids, C_4–C_{18}, and 0.4 equivalents of perchloric acid based on one equivalent of fatty acid, a number of new complex estolides were synthesized. Effects of two temperatures, 45 and 55°C, were investigated. Stirring was accomplished with an overhead motor and stir shaft. The perchloric acid-catalyzed reactions were influenced by the degree of mixing because of the heterogeneous nature of the reaction. Initial studies showed that reaction rates for the formation of estolides were slow and yields were lower over a period of 24 h when magnetic mixing was used. Longer reaction times were enough to obtain the same yield, but only at a cost of increased Gardner color. The effect of mixing on reaction rate was also observed by Showell *et al.* (13), who measured the rate of γ-stearolactone formation from the isomerization of oleic acid with perchloric acid.

The yields of estolides ranged from 45 to 65% after isolation by Kugelrohr distillation under the conditions described above. There was a noticeable difference in yield between reactions run at 45 vs. 55°C; the general trend was higher estolide yields with lower temperature (Table 1, entries K and L). At the higher temperature the carbocation migration was greater, thus the competition for the production of lactone was

TABLE 3
Monomer Data[a]

Monomer	Monomer mp[b] (°C)	Lactone δ/γ[c]	% *trans*[d]
A	Liquid	3.7	46.2
B	Liquid	1.8	41.7
C	Liquid	4.9	30.1
D	Liquid	2.7	37.9
E	Liquid	1.3	29.9
F	Liquid	1.1	32.9
G	Liquid	4.4	38.5
H	Liquid	1.6	39.2
I	25–27	5.1	43.2
J	23–24	2.2	47.4
K	37–39	4.4	45.3
L	36–37	1.6	48.7
M	51–52	5.0	49.3
N	48–51	3.9	47.6
O	57–60	2.9	51.8
P	58–59	1.3	66.0

[a]All data based on distilled monomer.
[b]Liquid at 22°C.
[c]Ratio determined by GC (SP 2380, 30 m × 0.25 mm i.d.).
[d]% *trans* determined by infrared analysis of the *trans* absorption band at 967 cm^{-1}. For abbreviation see Table 1.

greater. In general for products from the 55°C temperature reactions, monomer fractions obtained *via* Kugelrohr distillation contained a significant amount of lactone (Table 3). Showell *et al.* (13) demonstrated that oleic acid treated with one equivalent of perchloric acid at 85°C produced lactones in 88.2% yield and estolides in 11.8% yield. Our experiments were conducted at temperatures far less than 85°C in order to help minimize lactone formation and increase the yield of estolide.

The analysis and characterization of the free estolides by HPLC, GC, and NMR methods were far more complex than for traditional oleic estolides synthesized by Isbell and Kleiman (9). In the synthesis of free estolides **3** (Scheme 3) some unique structural possibilities were encountered. The EN in Table 1 demonstrated the amount of oligomerization for each set of estolides. Figure 1 shows the impact of the chain length of the saturated material on the EN for the reactions at 45°C. As the chain length increased, the EN decreased most likely due to steric or pK_a effects. For example, the larger saturated fatty acids could have inhibited the reaction once they added to the estolide, limiting the propagation of estolide formation from the acid end. Solution acidity also could have affected EN. Since pK_a values increase slightly with chain length, shorter-chain fatty acids have lower pK_a values and are therefore more acidic. Isbell *et al.* (21) demonstrated that as the amount of acid present in an estolide reaction increased the EN also increased. So, as the acidity of the solution increased there should have been an increase in the EN of the estolides, which was observed. The EN was also found to depend on temperature. As the temperature increased to 55°C, EN decreased for the individual free estolides, but the general trend observed at 45°C remained.

The estolide free acid **3** (Scheme 3) is shown as a saturated, capped estolide with an IV of zero. In practice, however, the IV for the reactions in Table 1 are in the teens. These higher-than-expected IV suggest that not all the estolides are capped with saturated material; the free acid estolides **3** contain some unsaturated oleic materials. Isbell *et al.* (21) reported that oleic estolides with comparable estolide numbers have IV around 35. An IV of 15 is close to that of a completely oleic-based estolide previously synthesized by Isbell after it was partially hydrogenated. The saturated capped estolides have an advantage over these partially hydrogenated estolides. The capped estolide reaction involves one step and inexpensive reagents as compared to two steps and expensive reducing metals. Since fewer alkenes are present in the final capped estolides, the oxidative stability should be greater than standard oleic estolides if the trends described by Akoh (24) hold true.

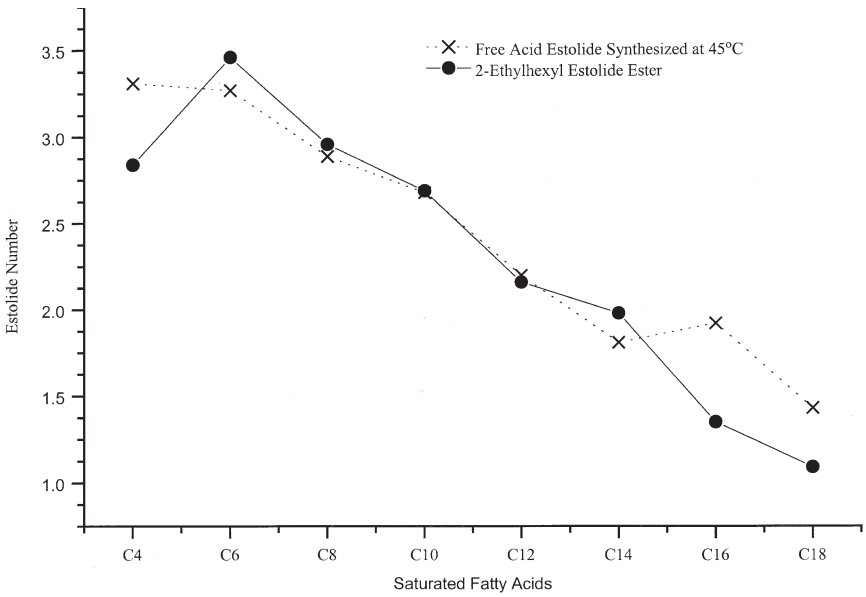


FIG. 1. Effects of saturated estolides vs. estolide number.

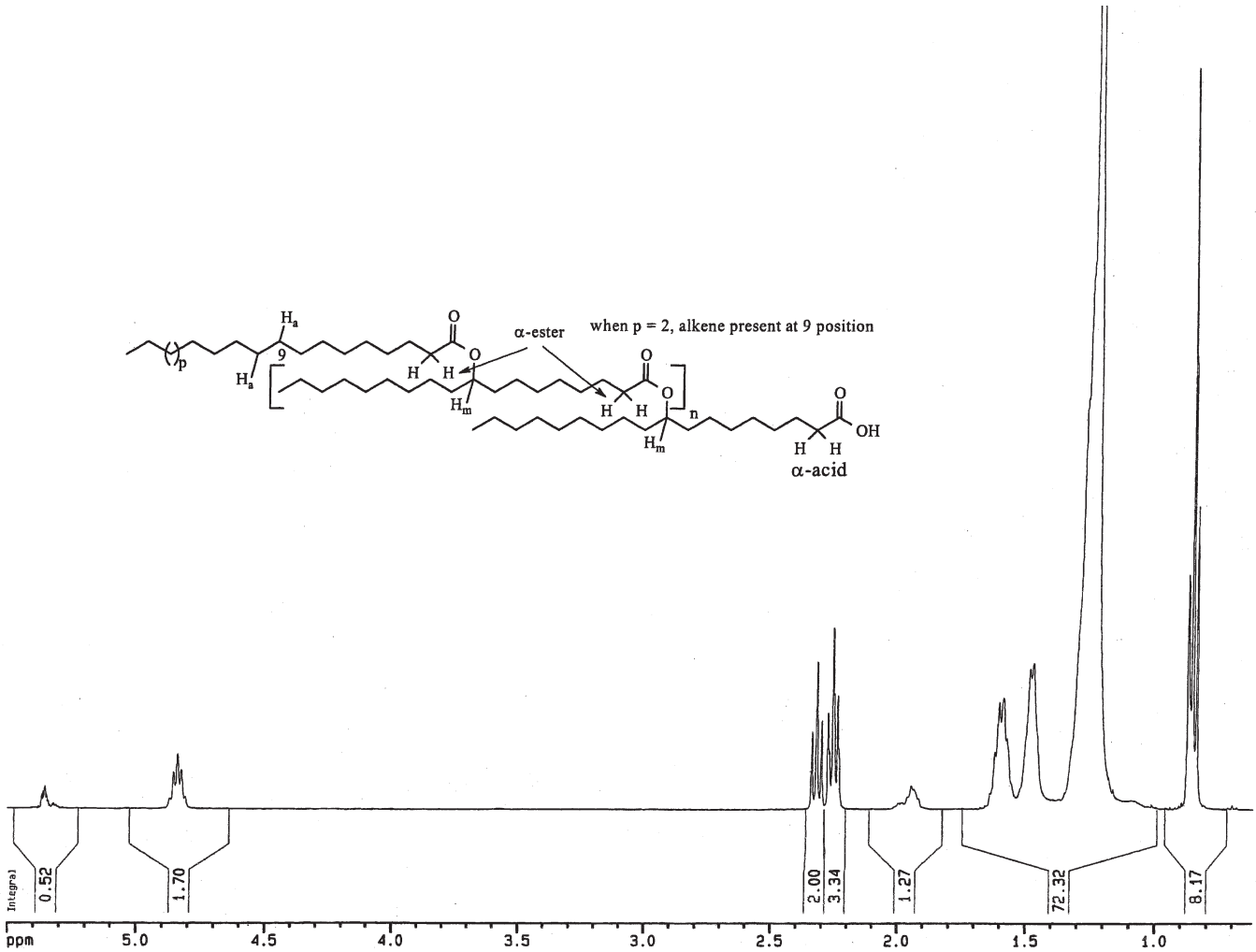


FIG. 2. Proton nuclear magnetic resonance (400 MHz) spectrum of estolide M from Table 1.

NMR characterization of estolides. The proton NMR spectra for the free estolides in Figure 2, specifically estolide M, show some key features of a typical estolide. The ester methine signal (H_m) at 4.84 ppm is indicative of an estolide linkage. Another distinctive feature is the α-methylene proton shift (2.32 ppm) adjacent to the acid and the α-methylene proton shift (2.25 ppm) adjacent to the ester. Integration of these signals provides a ratio for the number of ester bonds to acid functionalities. This ratio of α-ester/α-acid can be used as another means to determine the EN that is complementary to the GC method. The NMR indicates some presence of alkene in the estolide by the appearance of an alkene signal (H_a) at 5.36 ppm. The alkene signal indicates that some of the estolide is capped with unsaturated material, i.e., oleic acid. The alkene signal in the proton NMR supports the IV determined by GC, as the intensity of the NMR signals is comparable to the reported IV.

The carbon NMR spectrum contains the expected estolide signals. There are two different carbonyl signals present at 179.2 ppm (acid) and 173.7 ppm (estolide ester). The other distinctive signal is the methine carbon at 74.1 ppm, which is common to esters. These major peaks in the carbon NMR were also confirmed by a distortionless enhancement by polarization transfer (DEPT) experiment. The alkene carbons are only slightly noticeable, as these signals are about the same as the signal-to-noise ratio.

The conversion to the 2-ethylhexyl ester, M-2EH (Table 2), gives some predictable signal changes in the proton NMR. The α-carbonyl methylene protons have similar shifts, resulting in a multiplet from 2.27–2.22 ppm. As before, the alkene signal is noticeable at 5.35 ppm, confirming the IV determined by GC. The carbon NMR signals are indicative of the 2-ethylhexyl ester and are confirmed by a DEPT experiment.

Monomer fractions. The monomers collected from each distillation were analyzed for the extent of geometrical isomerization that occurred during the course of the reaction (Table 3). In general when the same estolides were synthesized under similar conditions but at different temperatures (Table 3, monomers from estolides E and F), a greater amount of isomerization occurred at the higher temperatures. As the amount of *trans* isomers increased, the probability of the monomer becoming a solid increased. This phenomenon was also demonstrated by Isbell *et al.* (20) with monomers from oleic estolide.

We showed solidification was not inevitable, however. The % *trans* alkene (Table 3) in the monomer was within the solidification range, but only about half the samples contained solids. Monomers A–H (Table 3) contained a saturated, short-chain fatty acid that did not undergo reaction. These short-chain acids were normally liquids and were unaffected by geometrical isomerization since there was no alkene present. The amount of oleic acid present in the monomer was important as well as the melting point of the saturated material.

The monomer fractions contained the remainder of the saturated fatty acid, oleic acid residues, and side products of hydroxy fatty acids and lactones. GC analysis of the lactones and hydroxy fatty acids was straightforward and provided normal fatty acid separation on a polar column. The ECL values were used for identifying the lactones and hydroxy fatty acids, and the corresponding GC separation allowed determination of the δ/γ ratios for all monomer fractions. To determine the amount of additional hydroxy fatty acid in the reaction mixture, the methyl esters were prepared with H_2SO_4/methanol as previously described. There were a number of hydroxy fatty acids possible in the mixture because of migration of the double bond. The lactones in the monomer were opened to the hydroxy methyl esters, but Tulloch (29) and Isbell and Plattner (10) reported that upon injection into a hot injection port these compounds underwent spontaneous ring closure. We tested our procedures with authentic δ- stearolactone samples provided by Cermak and Isbell (14), and were able to detect the γ- and δ-stearolactones and hydroxy fatty acids by GC analysis.

Monomer M (Table 3) contained 70% palmitic fatty acid, 7.2% hydroxy fatty acids, and 0.9% γ- and 4.5% δ-stearolactones as determined by GC analysis. As Showell *et al.* (13) demonstrated, as the reaction temperature increased, the δ/γ ratio decreased. The monomer fractions from the estolides supported this finding: the higher temperature reactions gave a lower δ/γ ratio (Table 3). The amount of lactone in the monomer fraction produced in any one reaction ranged between 6 and 23% of the total monomer.

Color. One of the most important physical properties to a consumer is the color of the oil. As a potential hydraulic fluid, the estolides need to meet the color requirements of the current useful hydraulic fluids.

The free estolides synthesized at 45°C had relatively low color (Table 1). When the reactions were repeated at 55°C, the colors of the estolides turned somewhat darker in every case, usually by two Gardner units. Esterification of the estolide to the 2-ethylhexyl esters caused additional problems, as the resulting Gardner colors were even darker (Table 2) than the free estolides (Table 1). This increase was expected, as the estolides were subjected to continued acidic conditions and higher temperatures, 60°C, than the nonesterified estolides. The estolide esters were decolorized with charcoal, and the colors improved anywhere from one to five Gardner color units. This decolorization step returned the estolide esters to nearly their original colors and left them commercially viable. The charcoal decolorization is one way to decolorize estolides; our laboratory has had success with other methods as well (30).

TABLE 4
A Sampling of Estolide Position as Determined by GC–Mass Spectrometry (MS) of Trimethylsilyl Ether of Alkali-Hydrolyzed Estolide[a]

Estolide fraction from Table 2	Hydroxy position	MS fragments carbonyl	MS fragments alkyl	Total abundance
Estolide (H-2EH)[b]	5	203	285	7.7
	6	217	271	19.9
	7	231	257	30.8
	8	245	243	35.9
	9	259	229	39.3
	10	273	215	40.0
	11	287	201	34.5
	12	301	187	27.5
	13	315	173	21.2
Estolide (I-2EH)[b]	5	203	285	2.6
	6	217	271	9.4
	7	231	257	20.3
	8	245	243	34.2
	9	259	229	54.2
	10	273	215	55.1
	11	287	201	34.4
	12	301	187	20.7
	13	315	173	11.7

[a]The same relative results were obtained for all complex estolides.
[b]Estolide is 2-ethylhexyl ester from Table 2. For other abbreviation see Table 1.

Esterification with 2-ethylhexyl alcohol. All the free estolides from Table 1 were esterified to >99% completion. The EN remained fairly constant throughout the series of reactions, as shown in Figure 1. This consistency indicated that there was very little transesterification taking place during the course of the reaction. The estolide esters were also evaluated for completion of esterification by examining the acid value. The acid values (Table 2) were well within the range of acceptability, <2 mg/g. By comparison, the free acid estolides from Table 1 had acid values around 35 mg/g.

GC–MS of silylated hydroxy esters obtained from hydrolysis of estolides. Estolide samples from Table 2, entries H-2EH and I-2EH, were saponified in 0.5 M KOH/MeOH then esterified with 1 M H_2SO_4/MeOH to give the corresponding hydroxy and nonhydroxylated fatty esters. The isolated mixture of fatty esters was then silylated and analyzed by GC–MS (31). The main mass spectral features were *m/z* 371 ($M^+ - 15$, 1.2%), 73 (TMS^+, 100%), and a Gaussian fragment representing cleavage at the C–C bond adjacent to the silyloxy positions (masses 173 to 315). The fragments and abundances are summarized in Table 4 relative to their respective positions on the fatty ester backbone; the same relative results were obtained for all complex estolides. The estolide position was distributed from positions 5–13 with the original Δ9 and Δ10 positions having the greatest abundances in the mass spectrum. This method demonstrates that during the estolide reaction there is migration of the double bond. The intensity of a fragment in the mass spectrum is not solely dependent on concentration of a species in the mixture, but more so on ionization energies and charge stabiliza-

tions. Thus, the apparent Gaussian distribution of hydroxy positions derived from the estolide, though likely due to structural similarities, may not have represented the amount of each position in the mixture, but merely indicated its presence.

ACKNOWLEDGMENTS

Jason E. Adkins, Michelle R. Edgcomb, Dawn N. Symonds, and Melissa L. Winchell assisted in the synthesis of these estolides and the derivation of the estolides for further analysis. David Weisleder provided the NMR spectra.

REFERENCES

1. Isbell, T.A., H.B. Frykman, T.P. Abbott, J.E. Lohr, and J.C. Drozd, Optimization of the Sulfuric Acid-Catalyzed Estolide Synthesis from Oleic Acid, *J. Am. Oil Chem. Soc. 74*:473–476 (1997).
2. Isbell, T.A., R. Kleiman, and B.A. Plattner, Acid-Catalyzed Condensation of Oleic Acid into Estolides and Polyestolides, *Ibid. 71*:169–174 (1994).
3. Isbell, T.A., and R. Kleiman, Mineral Acid-Catalyzed Condensation of Meadowfoam Fatty Acids into Estolides, *Ibid. 73*:1097–1107 (1996).
4. Isbell, T.A., T.P. Abbott, S. Asadauskas, and J.E. Lohr, Biodegradable Oleic Estolide Ester Base Stocks and Lubricants, U.S. Patent 6,018,063 (2000).
5. Isbell, T.A., and S.C. Cermak, Development of a Vegetable Based Functional Fluid Using Estolide Technology, in Abstracts from the Association for the Advancement of Industrial Crops annual meeting, St. Louis, MO, October 15–17, 2000, pp. 12.
6. Plattner, R.D., K. Payne-Wahl, L. Tjarks, and R. Kleiman, Hydroxy Acids and Estolide Triglycerides of *Heliophila amplexicaulis* L.f. Seed Oil, *Lipids 14*:576–579 (1979).
7. Payne-Wahl, K., and R. Kleiman, Quantitation of Estolide Triglycerides in *Sapium* Seeds by High-Performance Liquid Chromatography with Infrared Detection, *J. Am. Oil Chem. Soc. 60*:1011–1012 (1983).
8. Achaya, K.T., Chemical Derivatives of Castor Oil, *Ibid. 48*:758–763 (1971).
9. Isbell, T.A., and R. Kleiman, Characterization of Estolides Produced from the Acid-Catalyzed Condensation of Oleic Acid, *Ibid. 71*:379–383 (1994).
10. Isbell, T.A., and B.A. Plattner, A Highly Regioselective Synthesis of δ-Lactones from Meadowfoam Fatty Acids, *Ibid. 74*:153–158 (1997).
11. Clutterbuck, P.W., γ-Hydroxystearic Acid, *J. Chem. Soc. 125:*2330–2333 (1924).
12. Shepherd, I.S., and J.S. Showell, The Mechanism of the Aqueous Perchloric Acid Isomerization of Oleic Acid to γ-Stearolactone, *J. Am. Oil Chem. Soc. 46*:479–481 (1969).
13. Showell, J.S., D. Swern, and W.R. Noble, Perchloric Acid Isomerization of Oleic Acid, *J. Org. Chem. 88:*2697–2704 (1968).
14. Cermak, S.C., and T.A. Isbell, Synthesis of δ-Stearolactone from Oleic Acid, *J. Am. Oil Chem. Soc. 77*:243–248 (2000).
15. Isbell, T.A., and S.C. Cermak, Method for the Development of δ-Lactones and Hydroxy Acids from Unsaturated Fatty Acids and Their Glycerides, U.S. Patent 6,022,982 (2000).
16. Isbell, T.A., and B.A. Steiner, The Rate of Ring Opening of γ- and δ-Lactones Derived from Meadowfoam Fatty Acids, *J. Am. Oil Chem. Soc. 75*:63–66 (1998).
17. Frykman, H.B., T.A. Isbell, and S.C. Cermak, 5-Hydroxy Fatty Acids from δ-Lactones and Alkyl Glucamines, *J. Surfact. Deterg. 3*:179–183 (2000).
18. Vermeer, R., and B. Harichian, Hydroxy Containing Glycamides, Low Foaming Detergent Compositions Comprising Such and a Process for their Manufacture, U.S. Patent 5,750,733 (1998).
19. Erhan, S.M., R. Kleiman, and T.A. Isbell, Estolides from Meadowfoam Oil Fatty Acids and Other Monounsaturated Fatty Acids, *J. Am. Oil Chem. Soc. 70*:461–465 (1993).
20. Isbell, T.A., R. Kleiman, and S.M. Erhan, Characterization of Monomers Produced from Thermal High-Pressure Conversion of Meadowfoam and Oleic Acids into Estolides, *Ibid. 69*:1177–1183 (1992).
21. Isbell, T.A., M.R. Edgcomb, and B.A. Lowery, Physical Properties of Estolides and Their Ester Derivatives, *Ind. Crops Prod. 13*:11–20 (2001).
22. Modak, S.N., and J.G. Kane, Studies in Estolides. I. Kinetics of Estolide Formation and Decomposition, *J. Am. Oil Chem. Soc. 42*:428–432 (1965).
23. Zoleski, B.H., and F.J. Gaetani, Low Foaming Railroad Diesel Engine Lubricating Oil Composition, U.S. Patent 4,428,850 (1984).
24. Akoh, C.C., Oxidative Stability of Fat Substitutes and Vegetable Oils by the Oxidative Stability Index Method, *J. Am. Oil Chem. Soc. 71*:211–216 (1994).
25. *Official and Tentative Methods of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, AOCS, Champaign, IL, 1994.
26. Cermak, S.C., and T.A. Isbell, Biodegradable Oleic Estolide Ester Having Saturated Fatty Acid End Group Useful as Lubricant Base Stock, U.S. Patent Application 09/490,360 (2000).
27. Lanser, A.C., and E.A. Emken, Comparison of FTIR and Capillary Gas Chromatographic Methods for Quantitation of *trans* Unsaturation in Fatty Acid Methyl Esters, *J. Am. Oil Chem. Soc. 65*:1483–1487 (1988).
28. Ansell, M.F., and M.H. Palmer, The Lactonisation of Olefinic Acids: The Use of Sulphuric and Trifluoroacetic Acids, *J. Chem. Soc.*:2640–2644 (1963).
29. Tulloch, A.P., Gas Liquid Chromatography of the Hydroxy-, Acetoxy- and Oxostearic Acid Methyl Esters, *J. Am. Oil Chem. Soc. 41*:833–836 (1964).
30. Frykman, H.B., and T.A. Isbell, Decolorization of Meadowfoam Estolides Using Sodium Borohydride, *Ibid. 76*:765–767 (1999).
31. Erhan, S.M., and R. Kleiman, Meadowfoam and Rapeseed Oil as Accelerators in Factice Production, *inform 3*:482 (1992).

[Received October 30, 2000; accepted March 29, 2001]