Mineral Acid-Catalyzed Condensation of Meadowfoam Fatty Acids into Estolides[1]

Terry A. Isbell* and Robert Kleiman

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

ABSTRACT: Meadowfoam fatty acids (83% monoenoic fatty acid), reacted with 0.01–0.1 mole equivalents of perchloric acid, gave 33–71% yield of estolide, an oligomeric 2° ester, resulting from self condensation. Equimolar amounts of perchloric acid to fatty acid failed to produce estolide but converted the fatty acids to a mixture of lactones, mainly γ-eicosanolactone. Temperature plays a critical role; higher temperatures (75–100°C), at the same acid concentration, provide lactones while lower temperatures (20–65°C) yield estolides. Lower acid levels (<0.1 mole equivalents) gave the best yields (~70%) at 65°C. The estolide and monomer were characterized by nuclear magnetic resonance, infrared high-pressure liquid chromatography, gas chromatography, gas chromatography/mass spectrometry. The estolide is a mixture of oligomers with an average distribution near 1.65 ester units. The ester linkages are located mainly at the original double bond positions but have some positional isomerization to adjacent sites in accord with carbocation migration along the alkyl chain. The residual double bond of the estolide was extensively isomerized from *cis* to *trans* and positionally along the chain. The distilled monomer is similar in structure to the unsaturated portion of the estolide with geometrical and positional double bond isomerization. In addition, a significant amount of cyclization of the fatty acids to lactone (~30%) had occurred.

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KEY WORDS: Docosanolactone, eicosanolactone, estolide, geometrical isomerization, meadowfoam fatty acids homopolymer, perchloric acid, polyestolide, positional isomerization, sulfuric acid, *trans* fatty acids.

Meadowfoam (*Limnanthes alba*) is a promising new crop that is grown mainly near the Pacific Coast of North America. Meadowfoam, a winter annual, is establishing a role as an alternative crop for seed grasses currently grown in this region. The seed oil is unique in that the triacylglycerol consists of a >95% mixture of fatty acids with carbon chainlengths greater than 18. The composition of the fatty acids is (~66%) 5-eicosenoic acid, (~16%) 5,13-docosadienoic acid and (~11%) 5- and 13-docosenoic acid (1). As in most natural fatty acids, all double bonds are *cis*.

Meadowfoam oil and its fatty acids have been utilized in the development of novel materials with potential use as industrial agents. The oil has been vulcanized, and the resultant factices exhibit good properties in rubber applications (2–4). The fatty acids have been converted to amides (5), dimer acids (6), and estolides (7).

The latter are unique oligomeric fatty acids that contain a secondary ester linkage on the alkyl backbone of the fatty acid. Estolides have been synthesized from castor oil fatty acids (8,9) and 12-hydroxystearic acid (10,11) under thermal or acid-catalyzed conditions. Lipases can also catalyze the homopolymerization of hydroxy fatty acids (12). However, the formation of estolides has been limited to fatty acids with preexisting hydroxyl groups until recent efforts in our group have converted monounsaturated fatty acids into estolides by using clay-catalyzed high-pressure techniques (7) or a mineral acid-catalyzed process (13).

The high-pressure clay technique with oleic acid or meadowfoam fatty acids provided modest yields (<30%) of monoestolide. In contrast, the mineral acid-catalyzed condensation of oleic acid provided good yields of estolide (70%) with the formation of many ester oligomers (average 2.65). The mineral acid-catalyzed process used for oleic acid estolide synthesis might prove useful in the development of meadowfoam estolide. In addition, the proximal location of the Δ5 unsaturation may aid in the process through stabilization of the intermediate carbocation. Therefore, we set out to synthesize meadowfoam estolides by mineral acid catalysis and determine the composition of the resulting estolide.

EXPERIMENTAL PROCEDURES

Materials. Meadowfoam fatty acids were obtained by hydrolysis of meadowfoam oil, provided by the Fanning Corp. (Chicago, IL) and the Oregon Meadowfoam Growers Association (Salem, OR). Perchloric acid, *bis*(trimethylsilyl)trifluoroacetamide (BSTFA) and 10% palladium on activated carbon were purchased from Aldrich Chemical Co. (Milwaukee, WI). Concentrated sulfuric acid was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ). Potassium hydroxide, methanol, hexane (for extraction and high-pressure liquid chromatography, HPLC) and acetone (for HPLC) were obtained from Fisher Scientific Co. (Fairlawn, NJ). Boron trifluoride/methanol complex (14% wt/vol) and fatty acid methyl

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*To whom correspondence should be addressed at New Crops Research NCAUR, ARS, USDA, 1815 North University St., Peoria, IL 61604..

esters (FAME) were purchased from Alltech Associates, Inc. (Deerfield, IL). Tetrahydrofuran (THF), monobasic phosphate and dibasic phosphate were obtained from EM Science (Gibbstown, NJ). Triphenylphosphine was purchased from Eastman Kodak (Rochester, NY). Filter paper was obtained from Whatman (Maidstone, England).

Instrumental analyses. HPLC analyses were performed on a Spectra-Physics 8800 ternary pump (San Jose, CA) with a Spectra System AS3000 autosampler/injector from Thermo Separation Products (Fremont, CA), coupled to an evaporative light-scattering detector (ELSD) from Varex (Burtonsville, MD). A Dynamax (250 mm × 4.6 mm, 60 Å, 8 μm) silica column, purchased from Rainin Instrument Co. (Woburn, MA), was used to separate the estolide reaction mixtures. Components were eluted from the column with a hexane/acetone 80:20 mixture 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 was 1.5 standard liters per minute, and peaks were integrated with Hewlett-Packard Chemstation (Palo Alto, CA). Retention times for eluted peaks were: polyestolide 2.8 min, tetraestolide 2.9 min, triestolide 3.1 min, diestolide 3.5 min, monoestolide 3.8 min, monomer 4.4 min, γ-eicosanolactone 4.6 min, and δ-eicosanolactone 4.9 min.

Gas chromatography (GC). GC was performed on a Hewlett-Packard 5890 Series II gas chromatograph with a flame-ionization detector and an autosampler/injector. Analyses were conducted on two columns: SP 2330, 30 m × 0.25 mm i.d. (Supelco, Bellefonte, PA) and CPSIL-5, 15 m × 0.25 mm i.d. (Chrompack, Bridgewater, NJ). Saturated C8–C30 FAME provided standards for calculating equivalent chain-length (ECL) values (14).

SP 2330 conditions for standard FAME analysis were: column flow 1.48 mL/min with a helium head pressure of 25 psi; split ratio 40:1; programmed ramp 180 to 250°C at 3°C/min with a 2-min hold at 250°C; injector and detector temperatures set at 250°C.

SP2330 analysis conditions for aldehydes, dialdehydes, and aldehyde–esters from ozonolytic cleavage of unsaturated FAME were gas flow, injector, and detector conditions as previously described; programmed ramp 50 to 250°C at 5°C/min with a hold of 5 min at 250°C.

CPSIL-5 analysis conditions were column flow 0.77 mL/min with 10 psi helium head pressure; split ratio 75:1, programmed ramp 170 to 250°C at 3°C/min with a hold of 2 min at 250°C; injector and detector temperatues set at 250°C.

CPSIL-5 analysis conditions for aldehydes, dialdehydes, and aldehyde–esters from ozonolytic cleavage of unsaturated FAME were gas flow, injector, and detector conditions as previously described; programmed ramp 35 to 250°C at 3°C/min.

GC/mass spectrometry (MS) was performed on a Hewlett-Packard 5890A GC with a 15 m × 0.25 mm i.d. DB-1 column (J&W Scientific, Folsom, CA) and a Hewlett-Packard 5970 mass selective detector. GC conditions: helium head pressure 5 psi; 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.

δ-Eicosanolactone **6** retention time 14.5 min; MS: *m/e* 310 (M^+, 2%), 292 (M^+–18, 12%), 99 ($C_5H_7O_2$, 100%).

γ-Eicosanolactone (**5**) retention time 15.2 min; MS: *m/e* 310 (M^+, 1%), 292 (M^+–18, 7%) and 85 ($C_4H_5O_2$, 100%).

Trimethylsilyl (TMS) ether of 20:0 hydroxy ethyl ester **9a** retention time 17.0 min; MS: *m/e* 413 (M^+–15, 0.3%), 383 (M^+–45, 6%), TMS fragments toward the carboxylic functionality (329, <1%; 315, 1%; 301, 4%; 287, 2%; 273, 4%; 259, 12%; 245, 35%; 231, 80%; and 217, 2%), TMS fragments toward the alkyl terminus (313, 0.3%; 299, 40%; 285, 19%; 271, 6%; 257, 2%; 243, 1%; 229, 2%; 215, 2%; and 201, 1%) and 73 (TMS+, 100%).

TMS ether of 22:1 hydroxy ethyl ester **9d** and **9e** retention time 20.9 min; MS: *m/e* 439 (M^+–15, 1%), 409 (M^+–45, 1%), TMS fragments toward the carboxylic functionality with alkene located ~Δ13 (245, 5% and 231, 11%), TMS fragments toward the alkyl terminus with the alkene located ~Δ13 (325, 4% and 311, 1%), TMS fragments toward the carboxylic functionality with alkene located ~Δ5 (369, <1%; 355, 3%; 341, 10%; 327, 9%; 313, 2%, and 299, <1%), TMS fragments toward the alkyl terminus with alkene located ~Δ5 (257, 1%; 243, 4%; 229, 12%; 215, 13%; 201, 7%; and 187, 4%) and 73 (TMS+, 100%).

TMS ether of 22:0 hydroxy ethyl ester **9b** and **9c** retention time 21.5 min; MS: *m/e* 441 (M^+–15, 2%), 411 (M^+–45, 3%), TMS fragments toward the carboxylic functionality (371, 1%; 357, 6%; 343, 22%; 329, 26%; 315, 11%; 301, 5%; 287, 2%; 273, 1%; 259, 2%; 245, 6%; 231, 14%; 217, 2% and 203, 1%), TMS fragments toward the alkyl terminus (355, <1%; 327, 4%; 313, 2%; 299, 1%; 285, <1%; 271, 1%; 257, 3%; 243, 10%; 229, 30%; 215, 32%; 201, 13%; and 187, 4%) and 73 (TMS^+, 100%).

Bis TMS ether of 22:0 dihydroxy ethyl ester **9f** retention time 25.4 min; MS: *m/e* 529 (M^+–15, <1%), TMS fragments toward the carboxylic functionality of the ~Δ5 TMS with an alkyl TMS group ~Δ13 (245, 10% and 231, 24%), TMS fragments toward the alkyl terminus of the ~Δ5 TMS ether with an alkyl TMS group ~Δ13 (415, <1% and 401, <1%), TMS fragments toward the carboxylic functionality of the ~Δ13 TMS with a carboxylic TMS group ~Δ5 (445, <1%; 431, 2%; 417, 2%; 403, 1%; and 389, <1%), TMS fragments toward the alkyl terminus of the ~Δ13 TMS ether with a carboxylic TMS group ~Δ5 (257, <1%; 243, 5%; 229, 13.3%; 215, 15%; and 201, 7%) and 73 (TMS^+, 100%).

Infrared spectroscopy (IR) for quantitation of *trans* unsaturated fatty esters was performed in a Perkin-Elmer 137 dispersive dual-beam spectrophotometer. The *trans* double bonds in the material were determined precisely as previously described (15) from the *trans* absorption band at 967 cm^{-1}. Calculations for the determination of percentage *trans* in a mixture were made from a polynomial fit (R = 0.9998) of a standard curve prepared with six standards from 0 to 100% *trans*.

Nuclear magnetic resonance (NMR). ^{1}HNMR and ^{13}C

NMR were performed on a Bruker ARX 400 with a 5-mm dual proton/carbon probe (400 MHz ^{1}H./100.61 MHz ^{13}C) with $CDCl_3$ as the solvent in all experiments.

^{1}H NMR of estolide **7** δ 5.47–5.22 (*m*, 2H), 4.88–4.78 (*m*, 1H), 2.31 (*t*, *J*=7.5, 2H), 2.24 (*t*, *J*=7.4, 2H), 2.10–1.87 (*m*, 4H), 1.72–1.05 (*m*, 55H), and 0.85 ppm (*t*, *J*=7.3, 6H). ^{13}C NMR of estolide (**7**): δ 179.9, 179.8, 179.7, 179.5, 179.3, 173.6, 173.4, 173.2, 131.8–127.9, 74.0–73.4, 35.8–31.6, 29.7, 29.6, 29.5, 27.2–26.4, 25.3, 25.1–24.1, 22.7, 20.4 and 14.1 ppm.

^{1}H NMR of monomer: δ 5.49–5.25 (*m*, 2H), 4.45 (*m*, 1H), 4.25 (*m*, 1H), 2.61–2.25 (*m*, 4H), 2.10–1.02 (*m*, 46H), and 0.85 ppm (*t*, *J*=7.1 Hz, 6H). ^{13}C NMR of monomer: δ 180.2, 180.1, 179.1, 177.0, 172.0, 132.0–127.5, 81.1, 80.7, 35.8–22.6, 18.5, and 14.1 ppm.

^{1}H NMR of δ-eicosanolactone **6**: δ 4.27–4.24 (*m*, 1H), 2.59–2.53 (*m*, 1H), 2.46–2.40 (*m*, 1H), 1.92–1.81 (*m*, 3H), 1.69–1.66 (*m*, 1H), 1.58–1.47 (*m*, 4H, 1.30–1.15 (*m*, 27H) and 0.86 ppm (*t*, 6.7 Hz, 3H). ^{13}C NMR of δ-eicosanolactone (**6**): δ 172.0 (*s*), 80.6 (*d*), 35.8 (*t*), 31.9 (*t*), 29.7 (*t*), 29.6 (*t*), 29.5 (*t*), 29.5 (*t*), 29.4 (*t*), 29.4 (*t*), 29.3 (*t*), 27.8 (*t*), 24.9 (*t*), 22.7 (*t*), 18.5 (*t*) and 14.1 (*q*).

^{1}H NMR of γ-eicosanolactone **5**: δ 4.47 (*p*, *J*=7.4 Hz, 1H), 2.52 (*dd*, *J*=9.4 Hz, 2.5 Hz, 2H), 2.29 (*h*, *J*=6.5 Hz, 1H) 2.05–1.00 (*m*, 31H) and 0.87 ppm (*t*, *J*=6.2 Hz, 3H). ^{13}C NMR of γ-eicosanolactone (**5**). δ 174.0 (*s*), 81.0 (*d*), 35.6 (*t*), 31.9 (*t*), 29.7 (*t*), 29.5 (*t*), 29.5 (*t*), 29.4 (*t*), 28.9 (*t*), 28.0 (*t*), 25.2 (*t*), 22.7 (*t*) and 14.1 (*q*).

Methods. Estolide experiments were carried out in constant-temperature reactors, 0.1 L or 0.5 L, connected to a thermostated circulating bath, maintained at ± 0.1°C of the desired set point. The reactions were performed by first equilibrating the fatty acid at the desired temperature for several minutes, followed by addition of an appropriate mineral acid. Acids were added, and reactions were mixed continuously by magnetic stirring.

Perchloric acid reaction. Meadowfoam fatty acids (200 g, 0.645 moles) were placed in a 0.5-L reactor and equilibrated at 65°C for 10 min. $HClO_4$ (2.5 mL of 70%, 0.032 moles) was added rapidly to the stirred fatty acid and caused immediate formation of a brown mixture. Stirring was maintained throughout the course of the reaction to ensure adequate mixing of the two phases. After 24 h, the crude reaction product was poured into a 1-L separatory funnel, and the perchloric acid layer was removed. Hexane (200 mL) was added to the separatory funnel, and the mixture was neutralized with 1 M Na_2HPO_4 (2 × 100 mL). THF was then added to break the resulting emulsion. The organic layer changed from brown to pale yellow during the 1 M phosphate wash. The estolide solution was then washed (2 × 100 mL) with an aqueous phosphate buffer (pH = 4.7, 129.7 g monobasic phosphate and 0.85 g dibasic phosphate in 1L H_2O). The organic layer was dried over Na_2SO_4, filtered, concentrated and Kugelrohr-distilled (120 to 160°C @ 0.2 torr) to yield 121 g of a dark-colored estolide **7**.

Effects of acid, acid concentration, and temperature on the formation of estolide. All reactions were performed in a 0.1-L constant-temperature reactor under the conditions listed in Tables 1 and 2. Studies were performed on 20 g of meadowfoam fatty acids. Yields were determined by normal-phase HPLC analysis of the quenched reaction mixture (workup previously described), followed by isolation of the estolide by Kugelrohr distillation to remove the monomer. Mass balance of the isolated estolide confirmed the HPLC analysis.

Initial rates were determined by removing a small aliquot from the reaction mixture and diluting immediately in hexane. The aliquots were analyzed by HPLC as described in the experimental section. The initial rate of estolide formation was based on the percentage change of estolide over time. The light-scattering detector does not provide a linear response over all concentration ranges. To compensate for the variability in detector response, a region of good linearity for

TABLE 1
Factors Effecting the Yield of Estolide

Entry number	Acid[a]	Mole equivalents of acid	Temperature (°C)	Time (h)	Percentage lactone[b]	Percentage estolide[b]
1	$HClO_4$	0.01	75	21	3	33
2	$HClO_4$	0.05	65	25	12	61
3	$HClO_4$	0.05	65	31	15	71
4	$HClO_4$	0.10	65	25	24	56
5	$HClO_4$	0.10	50	30	12	55
6	$HClO_4$	1.00	65	25	92	1[c]
7	H_2SO_4	0.05	65	71	1	1
8	H_2SO_4	0.10	65	71	2	9
9	H_2SO_4	1.00	65	71	52	28[d]
10[e]	$HClO_4$	0.05	65	24	5	74

[a]Acids used were 70% $HClO_4$ and 98% H_2SO_4.
[b]Yield determined by high-pressure liquid chromatography.
[c]This reaction had a maximum estolide content of 24% at 15 min.
[d]This reaction had a maximum estolide content of 41% at 7 h.
[e]Starting fatty acids for this entry only were 22:2 concentrate (70%).

TABLE 2
Factors Affecting the Extent of Oligomerization

Entry number	Mole equivalents of acid[a]	Estolide number[b]	Temperature	Time (h)	Yield (%)
1	0.05	1.65	65°C	26	60
2	0.10	1.19	50°C	20	22
3	0.50	1.69	50°C	24	57
4	0.50	1.35	50°C	12	52
5	0.50	1.12	R.T.[c]	52	41

[a]Acid used was 70% $HClO_4$.
[b]Estolide number determined by high-pressure liquid chromatography and confirmed by gas chromatography analysis of the hydrolyzed estolide.
[c]Room temperature.

the detector was determined through the development of standard curves for both meadowfoam fatty acid and estolide. After identifying a linear region, sample concentrations were maintained within this area to minimize effects due to the variability in detector response. In addition, a rate reaction was performed with octacosane as an internal standard. The change in estolide concentration with time was monitored through the use of a standard curve based on the internal standard. The initial rate, determined with or without an internal standard, was the same.

Equilibrium study between lactone and estolide. δ-Eicosanolactone (2.0 g, 6.4 mmoles) was treated with 0.05 equivalents of $HClO_4$ (0.019 mL, 0.3 mmoles) at 65°C with magnetic stirring in a constant-temperature bath for 24 h. Aliquots were removed over the course of the reaction and analyzed by HPLC.

γ-Eicosanolactone (5.0 g, 16.1 mmoles) was treated with 0.05 equivalents of $HClO_4$ (0.069 mL, 0.8 mmoles) at 65°C with magnetic stirring in a constant-temperature bath for 24 h. Aliquots were analyzed by HPLC.

Alkaline hydrolysis of estolide. Alkaline KOH/methanol hydrolysis as previously reported for the liberation of hydroxy esters from oleic estolide (16) was only effective in a sealed vial where the temperature of the reaction could reach 100°C. This method was used for small-scale (10 mg) rapid analysis of estolide mixtures by GC. In contrast, a larger-scale reaction of ~1 g at reflux temperature of methanol failed to completely hydrolyze the meadowfoam estolide. However, the following modified technique gave complete hydrolysis of the estolide. Meadowfoam estolide **7** (1.1 g, 2.2 mmoles) was refluxed in 7 mL of 1.0 M KOH/ethanol for 7 h. After this time, 15 mL of 1.0 M HCl/ethanol was added and reflux was continued for 10 min. The reaction mixture was allowed to cool and was then poured into a separatory funnel with 25 mL ethyl acetate. The organic layer was washed twice with 25 mL H_2O, dried over Na_2SO_4, filtered through #1 Whatman filter paper and concentrated *in vacuo* to yield 1.04 g of hydrolysis mixture. The normal fatty acid **8a–8d** and hydroxy fatty acid ethyl esters **9a–9f** were analyzed by GC.

The hydroxy fatty acid esters were then separated from the unsaturated fatty acid esters by flash column chromatography: solvent front, 250 mL of hexane/ethyl acetate 95:5, followed by 50 mL of 100% ethyl acetate. Forty tubes were collected, and thin-layer chromatography (hexane/ethyl acetate 95:5, silica) of these solutions indicated three distinct fractions. Fraction 1 (0.46 g) was unsaturated esters **8a–8d**, fraction 2 (0.57 g) was monohydroxy fatty esters **9a–9e**, and fraction 3 (0.04 g) was dihydroxy fatty esters **9f**. This assignment was confirmed by ECL values from GC and GC/MS analysis of the TMS derivatives of the hydroxy fatty esters.

TMS derivatization of hydroxy fatty esters. Hydroxy fatty acid esters **9a–9f** (~10 mg) were dissolved in two drops pyridine and 0.1 mL BSTFA. This solution was then placed in a sealed vial for 5 min at 60°C. After this time, hexane (1 mL) was added, and the resulting solution was washed 2 × 1 mL 5% H_2SO_4 solution and dried over Na_2SO_4. The hexane layer was filtered and placed in a sealed GC vial and injected onto the GC and GC/MS.

Ozonolysis of unsaturated fatty esters for double-bond location. Ozonolysis was performed as described in a previous publication (15) on 10-mg samples. The kugelrhor-distilled monomers and the isolated unsaturated fraction (**8a–8d**) from estolide hydrolysis were cleaved with ozone and analyzed on the polar SP 2330 and nonpolar CPSIL-5 GC columns under the conditions outlined above. Methyl oleate, methyl linolate, and meadowfoam fatty acids were also reacted with ozone and analyzed under the same conditions to serve as appropriate standards. GC/MS confirmed aldehyde, dialdehyde, and aldehyde–ester ECL assignments.

RESULTS AND DISCUSSION

Factors affecting the formation of estolide. Previous work (13) in our laboratory reported a 70% yield of polyestolide from a 1.0 mole equivalent perchloric acid-catalyzed condensation of oleic acid. In light of this result, we anticipated that meadowfoam fatty acids would form estolide in a similar fashion, possibly catalytically, due to the potential neighboring group participation by the carboxylic acid functionality. Initial attempts to synthesize meadowfoam estolides with a 1.0 mole equivalent of perchloric acid provided mostly lactones with smaller amounts of estolide (<30%). The observance of lactones in the reaction mixture was not surprising because Showell *et al.* (17) and Isbell *et al.* (13) have ob-

served the formation of lactone under similar conditions. Showell's work demonstrated that increased temperature and acid equivalents provided a good yield of γ-stearolactone from oleic acid. However, Isbell demonstrated that lower temperatures (20 to 50°C) provided a good yield of estolide, even when up to 2.0 mole equivalents of acid were used.

In light of these findings, a set of experiments was designed to optimize the formation of estolide, and these results are reported in Table 1. In all cases, 70% perchloric acid produced higher yields of estolide than concentrated sulfuric acid. Perchloric acid provided a catalytic conversion of meadowfoam fatty acids into estolide (entry 1–3, Table 1), which had not been demonstrated previously except for clay catalysts (7), where only moderate yields (<30%) of estolide were obtained. Increased reaction time at low acid levels provided improved estolide yields (entry 2 and 3, Table 1). Equimolar amounts of acid completely inhibited the formation of estolide and resulted in the formation of lactone (entry 6, Table 1). Temperature plays a critical role in the formation of estolide, with higher temperatures under the same acid concentration providing increased amounts of lactone (entries 4 and 5, Table 1).

Figures 1 and 2 are time-course plots for the formation of estolide catalyzed by perchloric and sulfuric acid. Initial rates also were determined for each reaction and reported accordingly. Low levels of perchloric acid (0.05 and 0.10 mole equivalents) provided similar rates for the formation of estolide. However, the 0.05-equivalent reaction continued to generate estolide beyond 60%, whereas the 0.10-equivalent reaction reached equilibrium. The 1.00-equivalent reaction had an initial burst of estolide (24%, Fig. 1), but it was subsequently consumed over the course of the reaction to yield 92% lactone (entry 6, Table 1).

A 1.00 equivalent concentrated sulfuric acid reaction provided 41% estolide at 7 h, but it was eventually converted to lactone after 71 h (Fig. 2). The 0.10 and 0.05 equivalent sulfuric acid reactions continued to produce small amounts of estolide over the course of the reaction, even though at a slow rate (Fig. 2).

In light of these results, a mechanism was postulated (Scheme 1) for the formation of estolide from the main fatty acid component of meadowfoam. Protonation of 5-eicosenoic acid **1** will yield carbocation **2**, which is stabilized by the carboxylic acid functionality in a six-membered ring transition state **3**. Subsequent capture of the stabilized carbocation **3** with a second fatty acid **1** will lead to the estolide **7**. However, competing carbocation reactions can reduce the yield of estolide by a ring closure pathway of stabilized cation **3** to δ-eicosanolactone **6**. Fortunately, stabilized cation **3** exists in an equilibrium with **6** to provide a steady-state concentration of **3**. However, a hydride shift of hydrogen H_b will give the five-membered ring stabilized cation **4**, which will rapidly close to γ-eicosanolactone **5**. Once **5** is formed, regeneration of cation **4** or **3** will not occur. In addition, ring closure to form γ-lactones is 100 times faster then ring closure to form δ-lactones (18). Furthermore, ring opening of the γ-lactone proceeds at a slow rate in comparison to the rapid ring opening of δ-lactone (19).

The existence of an equilibrium between δ-lactone and estolide was confirmed by subjecting an isolated fraction of δ-lactone (>90%) to the 0.05 equivalent perchloric acid reaction conditions at 65°C. The δ-lactone produced a 67% yield of estolide after 24 h, thus demonstrating the existence of an equilibrium between cation **3** and δ-lactone **6**. In contrast, the γ-lactone, when subjected to the 0.05-equivalent perchloric acid reaction at 65°C, failed to open even after 24 h.

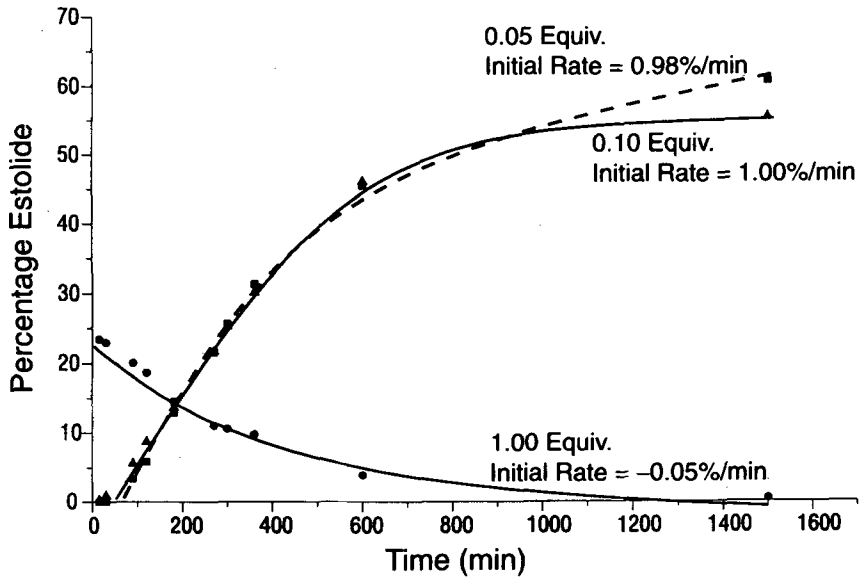


FIG. 1. Effect of perchloric acid on the rate of estolide formation. Rate determined by high-pressure liquid chromatography Dynamax silica (Rainin Instrument Co., Woburn, MA), 25-cm column.

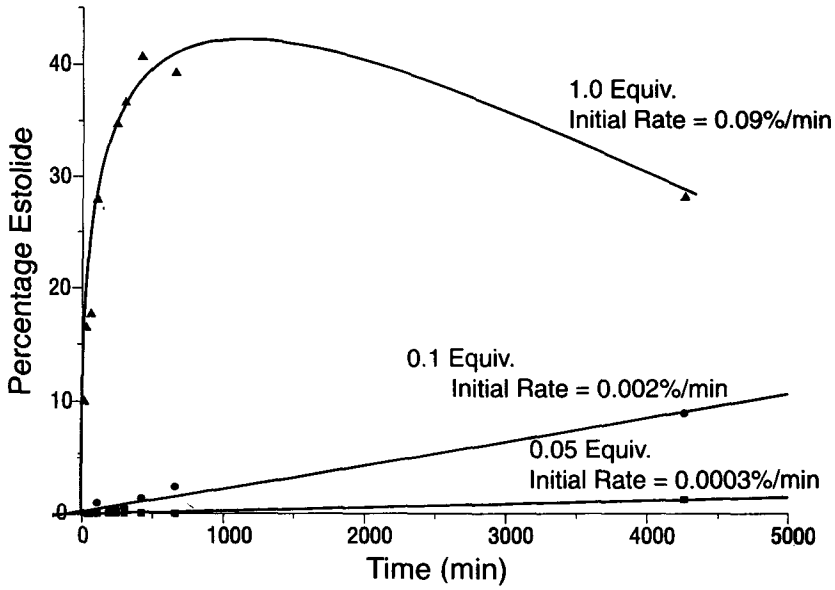
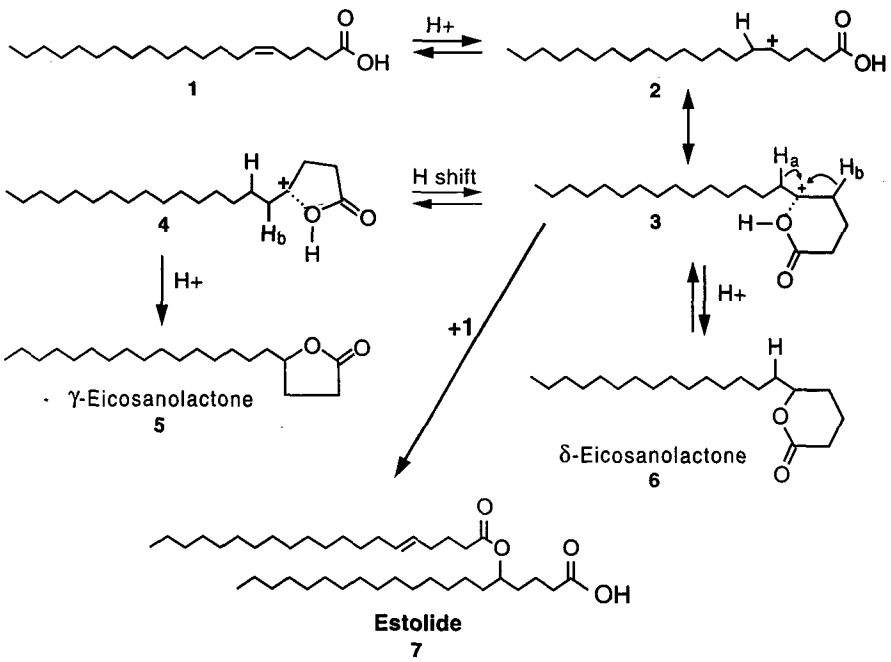


FIG. 2. Effect of sulfuric acid on the rate of estolide formation. Rate determined by high-pressure liquid chromatography Dynamax silica, 25-cm column. See Figure 1 for company source.



SCHEME 1

Factors affecting oligomerization. The extent of oligomerization of the estolide is affected by acid concentration, reaction temperature, and reaction time. Table 2 provides a summary of reaction conditions and their effects on polyestolide formation. The extent of oligomerization was determined by the ratio of hydroxy to unsaturated fatty esters found during GC analysis of the hydrolyzed estolide. The ratio of hydroxy to unhydroxylated esters is the estolide number (16). Higher acid concentrations and temperatures provide a larger extent of oligomerization (Table 2). Under constant temperature and stoichiometry, extended reaction times will yield larger estolide numbers.

Of particular note is the difference in the degree of oligomerization of meadowfoam fatty acids to that of oleic acid under similar reaction conditions. Estolides from meadowfoam fatty acids tend to yield monoestolide, whereas es-

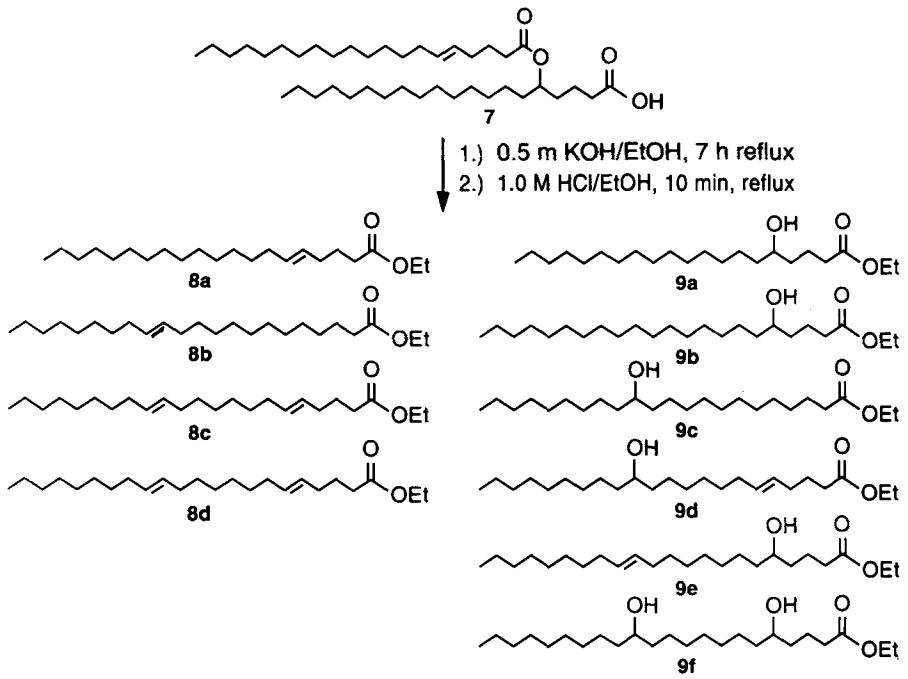
tolides from oleic acid yield many oiligomers with an average distribution greater then diestolide (16). This effect in meadowfoam for the formation of monoestolide is most likely due to the electronic repulsion of an approaching carboxylic acid by the carboxylic functionalities proximally (Δ5) located to the reactive carbocation site already in the estolide.

Estolides from 22:2 fatty acids, concentrate from meadowfoam. The 5,13-docosadienoic acid was isolated by crystallization from meadowfoam fatty acids by the procedure of Chang and Rothfus (20). This procedure gave 70% of the 22:2, which was then treated with perchloric acid under the conditions listed in entry 10 of Table 1 to yield 74% estolide. This material provided a slightly better yield of estolide then the original meadowfoam fatty acids and in a shorter period of time. The product had a larger extent of oligomerization than the meadowfoam fatty acid mixture with an estolide number of 2.2. This tends to support the previously mentioned electronic repulsion postulate because 22:2 has a Δ13 bond that is capable of forming estolide with less electronic repulsion from the carboxyl group for the approaching fatty acid. Hydrolysis of this estolide indicated not only the presence of monohydroxy compounds (43%) but also of dihydroxy compounds (13%), which resulted from two estolide moieties on one fatty acid backbone.

Characterization of estolide. The NMR of estolide **7** has several distinct features that are indicative of the estolide. The estolide methine proton resonates at 4.84 ppm, and two α methylenes are located at 2.31 ppm (α to the acid) and at 2.24 ppm (α to the ester). Both α methylenes are triplets, and integration of these two signals provides the estolide number (16). The main features of the ^{13}C NMR are the two carbonyl signals, 179.8 ppm for the acid carbonyl and 173.6 ppm for the estolide carbonyl. The estolide methine carbon has a chemical shift of 74.0 ppm.

Characterization of the hydroxy fraction from estolide hydrolysis. The normal-phase HPLC trace of the Kugelrohr-distilled estolide indicated 95.4% estolide and 4.6% residual lactones, δ and γ, in the mixture. In an effort to confirm the HPLC data for estolide oligomerization and determine the location of the ester linkage, alkaline ethanolic hydrolysis (Scheme 2) was performed. Of particular note, attempted hydrolysis in alkaline methanolic solutions at atmospheric pressure and methanol reflux temperature gave incomplete transesterification due to the poor solubility of estolide in methanol. This is in contrast to successful hydrolysis of the estolide in alkaline methanol hydrolysis in a sealed vial at 100°C. However, hydrolysis in refluxing ethanol, under atmospheric pressure, completely hydrolyzed the estolide.

Hydrolysis of the estolide provided hydroxy ethyl esters **9a–9f** and unsaturated ethyl esters **8a–8d** (Scheme 2). The hydrolysis mixture was treated with BSTFA silylating reagent and analyzed by GC. Integration of the eluted peaks from GC provided 42.5% hydroxy fatty esters, 39.5% unsaturated fatty esters, and 18% lactone. This indicates that hydrolysis of the 4- and 5-esters to the corresponding hydroxy acids resulted in cyclization to lactones under the hydrolysis reaction conditions or during the GC analysis. Other groups have failed to analyze 4- and 5-hydroxy acids by GC (21), but we have shown that the TMS ethers can be chromatographed with only small amounts of ring closure to the corresponding lactones. However, treatment of the lactone with silylating reagent did not provide 4- or 5-TMS derivatives. Failure of the lactones to derivatize with BSTFA indicates that the lactones must be forming in the hydrolysis reaction and not on column during



SCHEME 2

GC analysis. Therefore, the extent of ester bonds in the original estolide mixture is a ratio of the percentage hydroxy esters plus the percentage lactone divided by the percentage unsaturated esters.

Additional structural information could be gleaned about the estolide by separation of the hydrolysis mixture into unsaturated and hydroxy fractions, followed by subsequent derivatization and analysis. Separation of the estolide hydrolysis mixture was accomplished by flash-column chromatography into unsaturated esters **8a–8d**, monohydroxy esters **9a–9e** and dihydroxy esters **8a–8d**, monohydroxy esters **9a–9e** and dihydroxy esters **9f**.

The hydroxy and dihydroxy ester fractions were derivatized with BSTFA silylating reagent and analyzed by GC/MS. The GC trace of the monohydroxy fraction indicated a mixture of γ-eicosanolactone **5** (2.5%), δ-eicosanolactone **6** (17.8%), $C_{20:0}$ TMS derivative of **9a** (49.9%), $C_{22:0}$ TMS derivative of **9b** and **9c** (17.7%) and $C_{22:1}$ TMS derivative of **9d** and **9e** (12.2%). The mass spectra of the TMS derivatives of **9a–9e** are reported in Tables 3 and 4, and the TMS derivative of the dihydroxy fraction **9f** is reported in Table 5. The estolide position is distributed throughout the chain of the fatty acid backbone in a Gaussian fashion, with the majority of the positional isomers located at the double-bond positions found originally in the fatty acids of meadowfoam. This distribution is evidenced by the abundance column in Tables 3–5. The largest abundance of fragments around the TMS moiety occur at the 6, 13, and 14 positions. One would expect linkages of near-equal abundance for each position of the original olefins, positions 5, 6 and 13, 14. This expectation is nearly reflected in the data in Tables 3–5 with the exception of the low abundance at the 5 position. However, as was mentioned earlier, the 4- and 5-hydroxy acids spontaneously cyclize under the hydrolysis reaction conditions to form γ– and δ-lactones. Therefore, the 5-hydroxy position is represented by the δ-lactone in the GC/MS chromatogram, and the small amount of γ-lactone would be assigned to Δ4 estolides.

Characterization of the unsaturated fraction from estolide hydrolysis. The unsaturated fraction **8** from estolide hydrolysis (Scheme 2) was also examined for structural information by GC and IR. In addition, the olefins were ozonolytically cleaved, and quantitative analysis of the resulting aldehydes, aldehyde–esters, and dialdehydes was performed by GC and GC/MS to determine the extent of positional isomerization.

Table 6 provides a summary of the composition of the unsaturated fraction **8** from meadowfoam estolide hydrolysis. This material nearly parallels the original fatty acid distribution of meadowfoam but has a slightly increased percentage of 22:1 and 22:2. In addition, small amounts of lactones were found within the fraction, suggesting an incomplete separation by column chromatography of the unsaturated and hydroxy fraction.

The hydrolyzed fraction **8** was analyzed for geometrical isomerization by measuring the *trans* absorption band at 967 cm^{-1} in the IR spectrum (15). This material was found to be 72.0% *trans*. Fraction **8** is 91% unsaturated (Table 6), therefore 79% of the double bonds are *trans*.

Ozonolysis of the unsaturated fraction **8a–8e** provided information about the location of the residual olefin within the estolide. The original double bonds of meadowfoam have been distributed throughout the chain, with the largest percentage of the olefins encompassing the original positions; these results are summarized in Table 7.

TABLE 3
Estolide Position as Determined by Gas Chromatography/Mass Spectrometry (GC/MS) of Trimethylsilyl Ether of Alkali-Hydrolyzed Estolide 9a–9e

Fraction	Hydroxy position	MS fragments (amu) Carbonyl	MS fragments (amu) Alkyl	Total abundance
20:0 (**9a**)	5	217	313	2.1
	6	231	299	119.5
	7	245	285	53.6
	8	259	271	18.8
	9	273	257	6.6
	10	287	243	4.0
	11	301	229	6.7
	12	315	215	3.3
	13	329	201	1.7
22:0 (**9b–9c**)	4	203	355	1.2
	5	217	341	1.6
	6	231	327	18.3
	7	245	313	8.4
	8	259	299	3.3
	9	273	285	1.4
	10	287	271	2.3
	11	301	257	7.8
	12	315	243	21.3
	13	329	229	55.8
	14	343	215	53.8
	15	357	201	19.0
	16	371	187	4.9

TABLE 4
Estolide Position as Determined by GC/MS of Trimethylsilyl Ether of Alkali-Hydrolyzed Estolide. Monotrimethylsilyl Ether Monoene[a] 9e

Fraction	Hydroxy position	MS fragments (amu)		Total abundance
		Carbonyl	Alkyl	
22:1 (~Δ13)	6	231	325	15.8
(**9e**)	7	245	311	5.9
22:1 (~Δ5)	11	299	257	1.4
(**9d**)	12	313	243	6.3
	13	327	229	20.7
	14	341	215	22.7
	15	355	201	10.6
	16	369	187	4.7

[a]See Table 3 for abbreviations.

TABLE 5
Estolide Position as Determined by GC/MS of Trimethylsilyl (TMS) Ether of Alkali-Hydrolyzed Estolide Ditrimethylsilyl Ether[a] 9f

TMS ether location	Position	MS fragments		Total abundance
		Carbonyl	Alkyl	
Δ5	6	231	415	24.2
	7	245	401	10.7
Δ13	11	389	257	1.1
	12	403	243	6.3
	13	417	229	15.5
	14	431	215	17.1
	15	445	201	7.8

[a]See Table 3 for other abbreviations.

Table 7 provides normalized mole percentages of each of the respective fragments from reductive ozonolysis of several unsaturated materials. However, Table 7 does not directly report the olefin positions within the sample but rather lists the respective fragments from reductive ozonolysis. Attempted chromatographic separations (silica column, prep C_{18} and Ag+) of the individual 20:1, 22:1, and 22:2 portions of the unsaturated fractions of the monomer and fraction **8** were unsuccessful. As a result of the complex mixture, and the number of fragments created by ozonolysis, mathematical recombination of the original double-bond positions was not possible. However, a good summary of the bond distribution can be made by examining the individual components of the ozonolysis mixture when compared to the ozonolysis fragments of the starting fatty acids. Therefore, the aldehyde–ester fraction minus the dialdehyde fraction will indicate the general distribution of monoenes. Accordingly, the dialdehyde fraction represents the distribution for the degree of separation of the double bonds in the dienes. Lastly, the aldehyde fraction in combination with the dialdehydes and aldehyde–esters will confirm both the monoenes position and locate the position of the second double bond of the diene.

Characterization of monomers. The monomer portion of the estolide mixture is a material that has undergone chemical modification during the reaction but has not resulted in oligomeric material, such as estolide. The monomers were isolated from the estolide fraction by Kugelrohr distillation at 120–160°C @ 0.1–0.5 mm Hg.

NMR analysis of the monomer distilled from the meadowfoam estolide reaction gave resonances at 5.49–5.25 ppm, indicative of olefin protons. In addition, two signals (pentet at 4.45 ppm and multiplet at 4.25 ppm) indicated the presence of γ- and δ-lactones, respectively. The ^{13}C NMR confirmed this assignment with carbonyl signals of 180.2 ppm for fatty acid, 179.1 ppm for γ-lactone, and 177.0 ppm for δ-lactone. Methine signals were at 81.1 and 80.7 ppm for γ- and δ-lactone, respectively.

GC analysis of the monomer methyl esters is reported in Table 6. The original fatty acid composition of meadowfoam and the 22:2 concentrate is reflected in the monomer fractions. Hydrogenation of the monomer methyl esters gave normal-chain FAME and lactones with no evidence of branched fatty acids, in contrast to earlier meadowfoam estolide processes (15). However, lactone concentrations were nearly doubled under this process with nearly equal amounts of γ- and δ-lactones.

Ozonolytic cleavage, as mentioned above, of the monomers and starting fatty esters were analyzed by GC and GC/MS and are reported in Table 7. Double bonds migrated nearly throughout the fatty-acid chain in accord with a carbocation mechanism shown in Scheme 1. As expected, the monomer fraction and the unsaturated material **8**, obtained from estolide hydrolysis, are nearly identical.

TABLE 6
Characterization of the Monomers from the HClO4 Reaction by GC[a]

Assignment	Meadowfoam	22:2	Monomer from meadowfoam FA	Monomer from 22:2 concentrate of meadowfoam FA	Unsaturated fraction from hydrolyzed estolide
16:0	0.3	0.4	0.6	1.3	0.6
16:1	0.2	0.5	0.2	1.1	0.2
18:0	0.2	4.6	0.3	0.2	0.3
18:1	2.2	1.6	1.3	8.8	1.5
18:2	0.6	—	0.3	0.2	0.2
19:1	0.2	—	0.1	0.2	0.2
20:0	0.8	—	1.3	0.2	2.4
20:1	66.2	13.2	46.4	17.6	59.8
20:2	0.6	1.6	—	0.5	—
21:1	0.2	—	0.2	0.2	0.1
22:1	10.8	3.8	8.6	4.3	18.1
22:2	16.5	69.7	6.8	39.9	11.9
23:1	0.4	—	0.2	0.7	—
24:1	0.5	2.9	0.1	0.4	—
24:2	0.2	1.2	—	0.1	—
18:0 δ-Lactone	—	—	0.2	—	—
18:1 δ-Lactone	—	—	—	0.5	—
20:0 δ-Lactone	—	—	15.8	3.9	4.7
20:0 γ-Lactone	—	—	14.1	3.5	0.7
22:0 δ-Lactone	—	—	0.6	0.3	0.2
22:0 γ-Lactone	—	—	0.6	—	—
22:1 δ-Lactone	—	—	1.0	7.2	—
22:1 γ-Lactone	—	—	1.3	9.1	—
Other Hydroxy	—	—	1.3	1.0	—

[a]Normalized percentage; FA, fatty acid. See Table 3 for other abbreviation.

TABLE 7
Ozonolysis of Unsaturated Monomers and Hydrolyzed Estolide Fraction[a]

Chain	Meadowfoam			22:2 Concentrate[b]			Monomer[c]			Monomer from 22:2[d]			Hydrolyzed estolide[e]		
length	AE[f]	A[g]	DA[h]	AE	A	DA	AE	A	DA	AE	A	DA	AE	A	DA
4							13.6		6.8	10.6		2.7	16.5		4.4
5	90.7			87.5			37.2		7.2	32.9		7.1	36.8		6.4
6		1.7			5.9	1.0	23.9	1.2	12.3	19.8	4.2	14.4	22.0	1.3	13.7
7	0.6	0.6		0.8	2.6	1.1	9.8	2.7	22.1	8.8	8.8	20.7	8.7	2.9	21.5
8			98.4		1.0	95.6	3.9	5.9	21.8	5.5	17.7	26.6	3.2	6.5	24.0
9	1.9	26.4		5.8	78.6	0.8	1.8	7.4	16.6	5.1	22.2	16.4	1.3	8.5	17.0
10			1.6		0.4	1.5	1.2	6.1	8.8	4.4	17.2	7.4	1.0	6.6	9.2
11	0.9	0.4		3.1	1.3		1.5	3.8	3.7	3.9	9.0	3.2	1.4	3.7	3.8
12							2.2	4.4	0.8	3.2	4.7	1.1	2.7	3.9	
13	5.9	0.9		2.6	1.3		2.7	9.0		2.3	3.5	0.4	3.4	7.7	
14		0.4			0.4		1.4	19.0		2.9	4.2		2.4	18.2	
15		66.7		0.2	8.1		0.7	28.1		0.6	5.7		0.4	26.8	
16		0.2					0.3	10.4			1.9			11.4	
17					0.3			1.5			0.9			1.7	
18								0.3			0.04			0.6	

[a]Normalized percentage within each fragment.
[b]22:2 Concentrate isolated by crystallization from meadowfoam fatty acids.
[c]Monomer from 0.05 mole equivalent reaction.
[d]Monomer from estolide produced from 22:2 concentrate.
[e]Unsaturated fraction from hykdrolysis of estolide.
[f]AE = aldehyde esters.
[g]A = aldehyde.
[h]DA = dialdehyde.

The extent of geometrical isomerization for the monomers was determined by IR as previously mentioned. The meadowfoam-derived monomer contained 43.6% *trans* double bonds in the mixture. Because the monomer is 60.7% unsaturated (Table 6), the extent of geometrical isomerization to *trans* olefins is 71.8%. In a similar fashion, the 22:2 concentrate-derived monomer mixture contained 63.2% *trans* double bonds; 72.8% unsaturation present in the mixture yields 86.8% *trans* olefins.

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