Quantitation of Estolides by Fourier Transform Infrared Spectroscopy

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ABSTRACT: Estolides were produced from meadowfoam oil fatty acids, oleic, linoleic, petroselinic, and *cis*-5,*cis*-13 docosadienoic acids. Estolide reaction mixtures were quantitated by Fourier transform infrared spectroscopy and compared to the area percentages determined by high-performance liquid chromatography. The absorbance frequency of estolide carbonyl (1737 cm^{-1}) is different than the lactone carbonyl (1790 cm^{-1}) and the acid carbonyl (1712 cm^{-1}). Estolide standards were obtained by wiped-film molecular-still distillations and column chromatography.

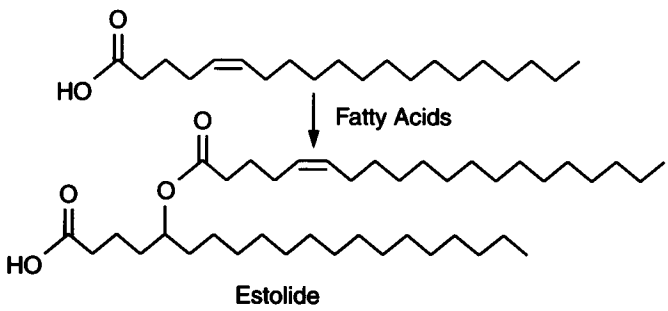
JAOCS 73, 563–567 (1996).

KEY WORDS: Estolide, FTIR, meadowfoam oil fatty acids, vegetable oil fatty acids.

Estolides (shown in Scheme 1) are formed when the carboxylic acid group of one fatty acid forms an ester bond with either a hydroxyl group or a double bond on the carbon chain of a second fatty acid (1–4). They can be produced by changing the reaction conditions of dimerization reactions (5,6):

dimerization reaction: 2 fatty acid ----- dimer + monomer + trimer
65% 30% 5% [1]

estolide reaction: 2 fatty acid ------ estolide + monomer + dimer
20% 79% 1% [2]



Estolide

SCHEME 1

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Estolides have potential uses in lubricants, greases, plasticizers, printing inks, and cosmetics. An accurate method of analysis will enable the reaction to be closely monitored and to make changes at desired times to shift the delicate esterification balance in favor of the desired products. Fourier transform infrared spectroscopy (FTIR) spectrometers are useful tools for this purpose because they have high signal-to-noise ratios, extensive data manipulation capabilities, such as subtraction and deconvolution of spectra, and can be used for continuous on-line monitoring of industrial processes (7).

EXPERIMENTAL PROCEDURES

Materials. Meadowfoam oil was obtained from Oregon Meadowfoam Growers Association (Salem, OR) and was split with high-pressure steam by Witco Corporation (Humko Chemical Division, Memphis, TN) to obtain fatty acids. Oleic and linoleic acids were purchased from Eastman Kodak Company (Rochester, NY). Petroselinic acid was isolated from carrot seed oil, and *cis*-5,*cis*-13 docosadienoic acid was previously isolated from meadowfoam oil (8). All other chemicals were reagent-grade. The fatty acid compositions of these fatty acid mixtures are given in Table 1.

Methods. The isolation and purification of estolide samples were obtained by (i) vacuum distillation and column chromatography. A crude reaction mixture that did not contain dimer acids was distilled at 160°C at 0.2 Torr pressure with a Kugelrohr apparatus (Aldrich, Milwaukee, WI) to remove the monomer fraction. The residue from this distillation was distilled, at 0.001 Torr pressure, on a wiped-film molecular still apparatus (KD1) from UIC (Joliet, IL). The first distillation was done at 170°C. The residue from this distillation was distilled again at 170°C to remove trace amounts of monomer, and the residue from the second distillation was distilled at 225°C. High-performance liquid chromatography (HPLC) analysis showed 100% estolide for the distillate fraction of the 225°C distillation.

Column chromatography was performed on a 40 cm × 2.5 cm glass column, packed with silica gel, type 60 A (Mallinckrodt Inc., Paris, KY). Monomers were eluted with 100% hexane, and estolides were eluted with a solvent mixture of 70% hexane, 29% ethyl acetate, and 1% acetic acid mixture. Under these conditions, any remaining polymeric

TABLE 1
Fatty Acid Compositions of Mixed Acids

Fatty acids[a]	Commercial oleic acid	High-oleic acid sunflower oil	Meadowfoam oil	Commercial linoleic acid	Petroselinic acid[b]	22:2 Concentrate from meadowfoam oil
Other acids	4.2	2.3	1.1	4.5		3.3
14:0	3.2					
16:0	6.2	3.6	0.5	3.2	6.0	
$16:1^{9}$	4.3					
18:0	2.1	4.7		0.9	1.3	0.9
$18:1^{6}$					92.0	
$18:1^{9}$	72.6	78.5	1.4	22.4	0.6	3.5
$18:2^{9,12}$	7.1	10.9	0.5	61.4	0.1	1.8
$18:3^{9,12,15}$	0.3			7.6		1.6
20:0			0.5			8.3
$20:1^{5}$			64.0			3.9
$22:1^{5}$			3.0			0.5
$22:1^{13}$			10.0			3.6
$22:2^{5,13}$			19.0			72.6

[a]Superscript number indicates the position of the double bonds on the carbon chain.
[b]Obtained from carrot seeds.

fractions remain on the column. The purity of the factions was determined by thin-layer chromatography (TLC), on silica gel coated plates (Whatman, Clifton, NJ) with a solvent system of hexane, diethyl ether, and acetic acid (70:29:1).

Gas-chromatographic (GC) analysis was performed on a Hewlett-Packard 5890 instrument (Palo Alto, CA) equipped with a flame-ionization detector (FID). The injector and detector temperatures were set at 350°C. A temperature program of 200 to 390°C at 4°C/min and a 12.5-min hold at 390°C was used on the DB-1 15 m column which had a 0.25-μm inside diameter (J&W Scientific, Folsom, CA). Methyl esters were prepared with either BF_3 in methanol or diazomethane (Fig. 1).

HPLC analyses were performed on a Spectra-Physics 8100 extended LC System with autosampler (San Jose, CA), with a Whatman Partisil PXS 10/25 polar silica column (Clifton, NJ), and a column flow rate of 0.8 mL/min coupled to a Varex evaporative light-scattering detector (ELSD II; Rockville, MD) (Fig. 2). The ELSD used for HPLC analysis does not have a linear response to changing estolide concentrations in a mixture (9,10). Therefore, calibration curves prepared with meadowfoam oil fatty acids and oleic acid monoestolides were used. The detector nebulizing gas was nitrogen, and the drift tube was heated to 74°C with an exhaust temperature of 54°C and a flow rate of 55 mL/min. For isocratic systems, a mobile phase of 50% dichloromethane and 50% dichloromethane/methanol/ acetic acid (98:2:0.25) was used.

FTIR analysis was done in a 0.0541-mm, fixed-pathlength cell with CaF_2 windows in a Mattson SIRIUS instrument (Madison, WI), equipped with an MATTSON FIRST analytical software program. Samples were weighed to 50.0 ± 0.5 mg into a 3.0-mL vial with a Teflon-lined cap, and diluted with 0.95 mL of cyclohexane. The sample was transferred after dissolution into the IR cell with the aid of a syringe. The spectra were taken at 2 cm^{-1} resolution and 32 co-added scans per spectrum. The instrument was purged with nitrogen. After the crude spectrum was taken, a cyclohexane spectrum, taken

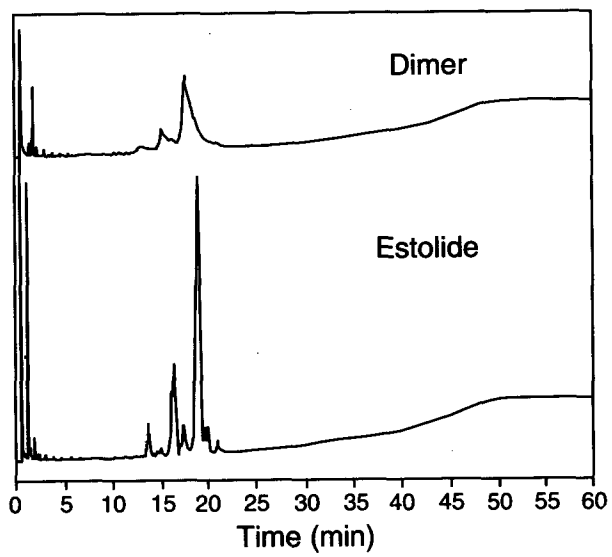

FIG. 1. Gas-chromatographic-chromatograms of estolides and dimer acids.

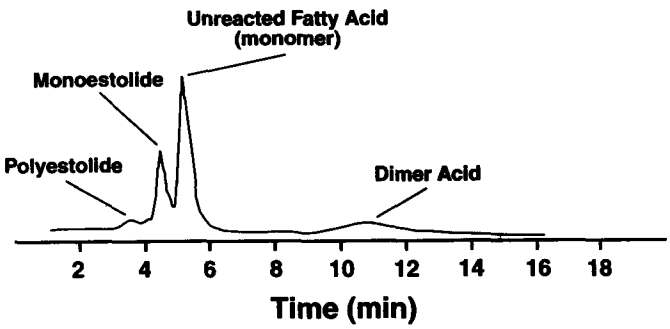

FIG. 2. The separation of the components of an estolide reaction mixture, containing dimer acids, on a high-performance liquid chromatographic instrument.

in the same cell, was subtracted from the sample solution by using a factor of 0.95. Water vapor was also subtracted until a smooth baseline resulted. The spectra were then baseline-corrected. Baseline separations of the 1737 cm^{-1} estolide and the 1712 cm^{-1} carboxylic acid carbonyl absorption peaks were obtained by using the deconvolution program between 1500 and 1900 cm^{-1} with an enhancement factor of 1.5 and a bandwidth at half height of 8 (Fig. 3). The percentage estolide was then calculated from absorbances, as seen in Equation 3:

$$\%\text{estolide} = \frac{A_{\text{sample}}(1737\ cm^{-1}) - A_{\text{baseline}} \times 100}{A_{\text{standard}}(1737\ cm^{-1})} \quad [3]$$

RESULTS AND DISCUSSION

The dimer acid fraction in Equation 1, can be separated from the monomer and trimer fractions by vacuum distillation (5). Estolides, on the other hand, distill at temperatures close to dimer acids. A complete separation, even after successive distillations on a molecular still, could not be achieved. They elute with dimer acids on a DB-1 GC column (Fig. 1).

Although GC and HPLC can give us quantitation of estolides, interferences by monomer, polyestolides, and dimer acids, if the baseline separation is not good, can contribute to inaccurate results. An infrared procedure was developed to obtain an independent method of quantitation to corroborate chromatographic methods. This was accomplished by using the differences in absorbances of the carbonyl stretch (Fig. 4). Therefore, the quantitation of estolides, which in this case is the sum of mono- and polyestolides, is obtained by infrared spectroscopy and the Beer's Law relation (Equation 4) between absorbance and concentration (11):

$$A = \varepsilon bC \quad [4]$$

A stands for absorption, ε is the molal extinction coefficient, b, is the pathlength, and C, concentration in molarity.

The estolide carbonyl group absorbs at 1737 cm^{-1}, whereas carboxylic acid and lactone carbonyl groups absorb at 1712 and 1790 cm^{-1}, respectively (Fig. 4). However, the maximum absorption of oleic acid with 18 carbons, and meadowfoam oil fatty acids, mostly 20 and 22 carbons long, are slightly different. According to Beer's law, absorbance is directly proportional with concentration, and concentration is inversely proportional with molecular weight. Oleic acid with a lower molecular weight in a weight percentage solution will have a slightly higher absorbance value. Therefore, in the calculation of percentage estolides from oleic, linoleic, and petroselinic acids, an oleic acid estolide standard was used. Meadowfoam oil fatty acid estolide standard was used in the percentage estolide calculations from *cis*-5, *cis*-13 docosadienoic acid and meadowfoam oil fatty acids.

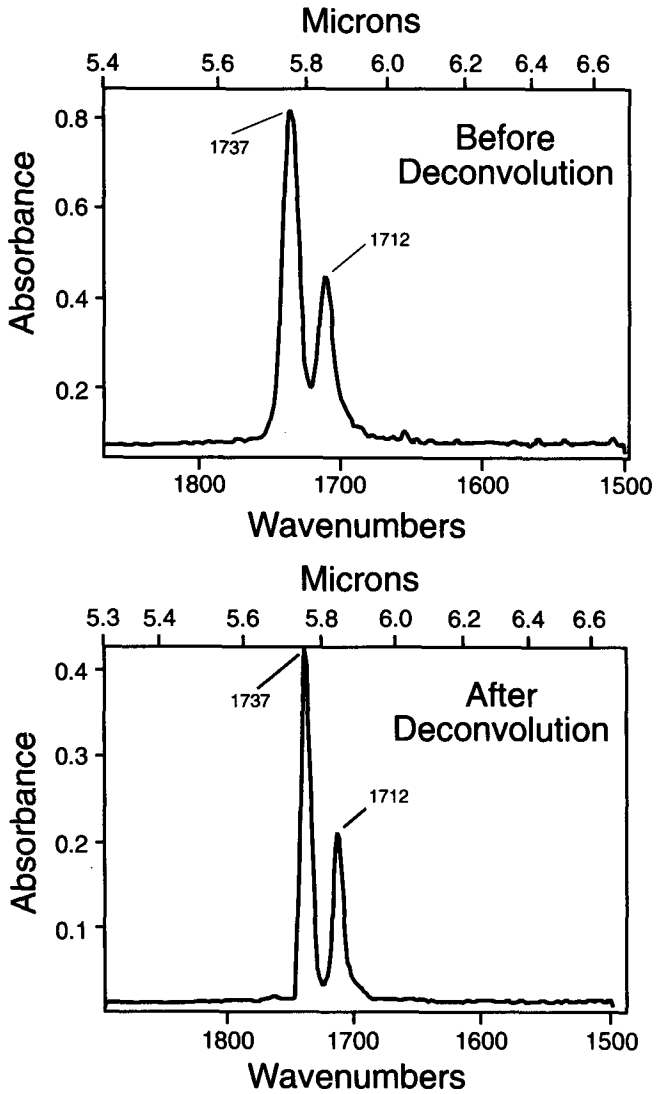

FIG. 3. Fourier transform infrared spectra before and after deconvolution of the estolide peak.

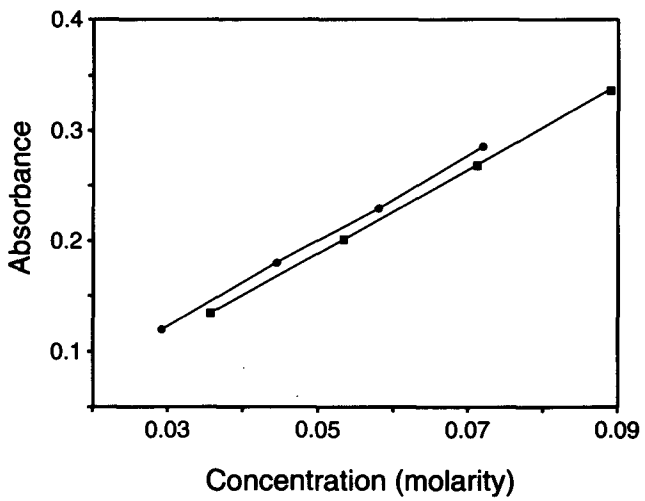

FIG. 4. Absorbances of components of a reaction mixture containing estolides and lactones on a Fourier transform infrared spectrum.

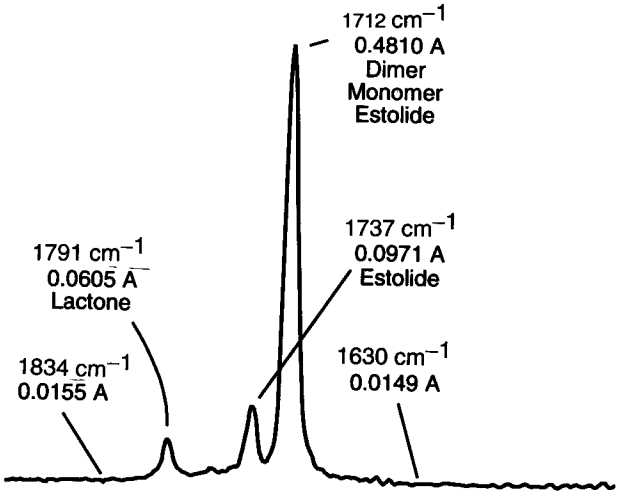

FIG. 5. The change of absorption of estolides, with changing concentration, in the Fourier transform infrared spectroscopy.

The calibration lines for oleic acid and meadowfoam oil fatty acid estolides are shown in Figure 5. Deviations from Beer's law are known at high concentrations (11), but in these cases the deviations were not significant.

There is good agreement between FTIR and HPLC estolide analyses (Tables 2 and 3). The differences in measurements between methods are negligible if uncertainties in sample preparations are considered. Quantitation of estolides in crude reaction mixtures by both methods are reported in Table 2. The estolide yield in a reaction mixture was monitored with time by both FTIR and HPLC analyses, and the results are reported in Table 3. In both cases, results indicate that either instrument can be used for the quantitation of estolides.

As mentioned earlier, dimer acids have the same GC elution times on a DB-1 column as estolides. There were some reaction mixtures that did not have dimer acids, as determined by HPLC. In these cases, the fractions were isolated by column chromatography, weighed, and analyzed for purity on a gas chromatograph and by TLC. In cases where the fraction contained other material besides estolides, the estolide percentage was determined by the area under the peak and converted into grams of estolide from the total weight of the sample. Estolide content, determined gravimetrically, was compared to the estolide amount determined by FTIR. The gravimetric results are slightly lower than results measured by the FTIR (Table 4). The lower values of the gravimetric-GC method is probably due to material loss during the column separation and to the uncertainties in cases where baseline separation between peaks of the GC chromatogram was not possible.

One drawback of the FTIR analysis system is its inability to differentiate monoestolides from polyestolides. However, in cases where reaction conditions produce mostly monoestolides, FTIR advantages will make it a useful tool to monitor estolide reactions.

TABLE 2
Estolide Percent Measured by Fourier Transform Infrared Spectroscopy (FTIR) and High-Performance Liquid Chromatography (HPLC)

Fatty acids	% Estolide by FTIR	% Estolide by HPLC
High-oleic sunflower oil fatty acids	14.30	13.80
cis-5,*cis*-13 Docosadienoic	56.72	56.76
Petroselinic	78.34	79.25
Meadowfoam oil fatty acids	82.90	83.50
Linoleic	82.14	82.50
Oleic	82.78	83.84

TABLE 3
Monitoring the Estolide Yield in a Continuing Reaction[a]

Sample	% Estolide by FTIR	% Estolide by HPLC
1	17.15	17.65
2	17.54	17.56
3	18.91	18.93
4	18.59	18.62
5	18.65	18.69
6	19.95	19.57

[a]Abbreviations as in Table 2.

TABLE 4
The Comparison of FTIR and GC-Gravimetric Methods in the Determination of Estolides in Mixtures[a]

	% Estolide	
Sample	By FTIR	By GC-gravimetric
1	82.78	81.46
2	82.20	80.23
3	79.20	75.44
4	56.72	54.83

[a]Abbreviation as in Table 2; GC, gas chromatographic.

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[Received June 9, 1995; accepted January 23, 1996]