Sequential Gas Chromatographic Procedure for Microanalysis of Monoenoic Double Bond Position in Hydrogenated Oils

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ABSTRACT

Quantitative cleavage of epoxyoctadecanoates with periodic acid (HIO₄) has been demonstrated and the technique incorporated into an all gas chromatographic system for detailed lipid analysis. The overall procedure involves three sequential gas chromatographic separations interspersed by two microreactions. By this procedure, a complete analysis for cis- and trans-geometric isomers corresponding to each positional octadecenoate isomer is obtained. Total sample requirements are less than 10 mg, and the elapsed analysis time/sample is less than 10 hr. In this all gas chromatographic procedure, a lipid methyl ester sample is first separated by preparative gas chromatography, and the monoene fraction is collected and epoxidized. Next, the epoxidized sample is separated by gas chromatography into cis- and trans-epoxyoctadecanoate fractions. These epoxyoctadecanoate fractions are collected and cleaved with HIO₄ into aldehyde and aldehyde-ester fragments, which are quantitatively analyzed by gas chromatography. The double bond positions are determined from the aldehyde and aldehyde-ester cleavage data, which are stored and processed by a computerized on-line gas chromatographic data acquisition system. The procedure was tested on pure octadecenoate isomers, standard mixtures, and commercially hydrogenated vegetable oils. Analyses of hydrogenated vegetable oils are compared with data acquired by reductive ozonolysis.

INTRODUCTION

Location of fatty acid double bond position is an important part in a wide variety of lipid researches. Double bond location is used widely in the analysis of hydrogenated vegetable oils, characterization of lipid extracts from biological sources, and for proving isotope and chemical purity of synthetic fatty acids. Lemieux and von Rudloff's original permanganate-periodate (1) oxidative cleavage method for double bond location was used for many years by lipid chemists (2,3) but has given way to ozonolysis procedures. Nickell and Privett used ozonolysis-pyrolysis to cleave octadecenoate standards (4) and Davison and Dutton (5) first applied ozonolysis-pyrolysis to isomeric mixtures of octadecenoates isolated from hydrogenated methyl linolenate. Stein and Nicolaides (6) demonstrated the reduction of ozonides to aldehydes with triphenylphosphine. Currently, ozonolysis followed by reduction with triphenylphosphine is used by many lipid laboratories for double bond location (7-9).

Recently, we described an epoxidation-gas chromatography (GC) technique for cis- and trans-analysis of octadecenoate isomers via the cis- and trans-epoxyoctadecanoate derivatives (10). We now are reporting upon the combination of this epoxidation-GC technique with a HIO₄ cleavage procedure (11,12) to provide for the first time an all GC approach for obtaining quantitative data on double bond location in cis- and trans-octadecenoate fractions from hydrogenated fats and oils. The combined epoxidation-HIO₄ procedure uses HIO₄ to cleave the epoxidized octadecenoate isomers into aldehyde and aldehyde-ester fragments. Subsequent analysis of these fragments allows the position of the oxirane ring to be determined.

Starting with the fatty acid methyl esters, the overall procedure involves three sequential GC separations and two microreactions. In this procedure, quantitative data are obtained for fatty acid composition, cis- and trans-configuration and double bond distribution.

The procedure has been demonstrated with pure octadecenoate isomers, octadecenoate mixtures of known composition, and commercial partially hydrogenated vegetable oil. The double bond distribution data are compared with data obtained by reductive ozonolysis.

EXPERIMENTAL PROCEDURES

Standards

Samples of methyl cis-6-octadecenoate were purchased from Hormel Institute; methyl cis-
15-octadecenoate (13) and methyl trans-9-octadecenoate (14) were prepared by previously published procedures. A mixture containing methyl cis-9-octadecenoate, methyl cis-12-octadecenoate and methyl cis-15-octadecenoate was prepared by hydrazine reduction of linolenic acid and analyzed by capillary GC (13,15).

**Commercially Hydrogenated Vegetable Oil**

A commercially available salad oil and shortening manufactured from partially hydrogenated vegetable oil were previously studied as samples A and E (16). Methyl esters of these samples were prepared by transesterification with methanol containing sodium methoxide as a catalyst.

**Periodic Acid (HIO₄)**

Periodic acid (HIO₄·2H₂O) assayed as 99.5% pure from Matheson Coleman & Bell, Elk Grove Village, Ill., was used in all of the experiments. The HIO₄ crystals were ground to ca. a 60 mesh powder for use in the HIO₄-Et₂O cleavage experiments. Since the powder is hygroscopic and photo unstable, exposure to atmospheric moisture and light must be kept at a minimum.

**Diethyl Ether Purification**

Commercial anhydrous reagent grade diethyl ether was found to require purification to prevent extraneous compounds from appearing during the HIO₄ cleavage reaction. The purification procedure consisted of shaking 200 ml ether with 10 g activated alumina (grade II) for 5 min, allowing the suspension to settle for 30 min and then decanting off the ether. The ether then was treated with a second portion of activated alumina and allowed to stand overnight at 3 C. The ether then was decanted off and treated with 5 g chromatographic grade silica gel and stored at 3 C until needed.

**Periodic Acid-Methylene Chloride (HIO₄-CH₂Cl₂)**

The HIO₄ used in the HIO₄-CH₂Cl₂ cleavage procedure was first dried for 16 hr at 100 C. The dried HIO₄ crystals were slightly yellow and easily ground to a 80-120 mesh powder. The methylene chloride was American Chemical Society reagent grade and did not require purification.

**Periodic Acid-Diethyl Ether Reagent (HIO₄-Et₂O)**

HIO₄-Et₂O reagent was used to cleave the epoxides directly. The HIO₄-Et₂O reagent was prepared by adding 2-3 ml purified ether to ca. 200 mg powdered HIO₄ and then shaking the mixture for 2-3 min to saturate the ether with HIO₄. The HIO₄-Et₂O mixture initially became cloudy and then cleared on standing at room temperature. The HIO₄-Et₂O reagent is stable for 24 hr, but best results were obtained when it was freshly prepared before each use.

**Preparative GC**

An octadecenoate fraction from commercially hydrogenated vegetable oil methyl esters was collected from an Aerograph Autoprep (model 600P), fitted with an aluminum 10 ft x 3/8 in. 10% EGSS-X column. The octadecenoate fraction was collected in glass tubes loosely packed with a 2 in. segment of glass wool. A single injection of 4-5 µliter sample generally provided enough octadecenoate (ca. 1.5 mg) for subsequent analyses.

**Epoxidation**

The collected octadecenoate sample was washed into a 2.0 ml vial with hexane, the solvent evaporated, and the residue (ca. 1.5 mg) epoxidized as previously described, using 100 µliter peracetic acid (10). The epoxidized octadecenoate mixture was diluted with 0.5 ml water and extracted twice with 0.2 ml portions of hexane. Combined hexane extracts were washed with an equal volume of water; the hexane layer was drawn off with a pipette, evaporated to dryness, and redissolved in 20 µliter hexane.

**GC Separation and Collection of Epoxides**

The cis- and trans-epoxyoctadecenoate isomers were separated and measured on an EGSS-X column as described previously (10), except that the Packard GC (model 6000) was modified with a 10:1 sample splitter at the exit of the column to allow collection of samples, since a flame ionization detector (FID) was used. The splitter assembly receiving the 10 parts of sample was heated to 230 C. Samples (0.2-0.3 µliter) of epoxidized octadecenoates dissolved in hexane were injected and the cis- and trans-epoxyoctadecenoate fractions collected in 3 in. segments of no. 17 gauge Teflon tubing attached to the sample splitter.

**Epoxide Cleavage (HIO₄-Et₂O) (12)**

Conical shaped vials were prepared by drawing out the bottoms of standard 0.5-dr screw-capped vials to 1-2 mm diameter. The collected epoxide fractions were washed from the Teflon tubing into these vials with 100 µliter hexane. The hexane was evaporated under a stream of nitrogen and the epoxides mixed with 50 µliter HIO₄-Et₂O reagent. The solution was allowed to stand at room temper-
FIG. 1. Gas chromatography (GC) of HI04 cleaved standards (a) cis-6,7-epoxyoctadecanoate; (b) trans-9,10-epoxyoctadecanoate; (c) cis-15,16-epoxyoctadecanoate. GC conditions: temperature programed from 50-240 °C at 4 °C/min and held for 30 min; 180 cm x 4 mm glass column packed with 30% mixture of OV 17-225.

FIG. 2. Gas chromatography (GC) of HI04 cleaved epoxidized 9-, 12-, and 15-octadecenoate mixture. GC conditions are the same as in Figure 1.

and FID. The aldehyde and aldehyde-ester cleavage products were separated on a column packed with a 50:50 mixture of OV 17 and OV 225 on 100-120 mesh Chromosorb WHP (8). The GC was temperature programed from 50-240 °C at 4 °C/min and at a helium flow rate of 30 ml/min.

The programing conditions did not produce extensive baseline drift at moderate detector sensitivities. Standard dual column techniques can be used to compensate for baseline drift, if column bleed becomes a problem when high sensitivities are necessary.

Computerized Data Processing

An IBM 1800 computer was interfaced with the gas chromatographs to provide real time on-line data acquisition. The GC data were integrated and processed by typical GC computer techniques using a modified McCullough IBM 1800 GC monitoring program (17).

RESULTS

All the separations and quantitative analyses used in this all GC procedure are performed by GC techniques. The necessary derivatives are prepared quickly and easily. Total sample requirements ranged from ca. 10-20 mg. The only step in the procedure which had not previously proven to be a quantitative technique was the HI04 cleavage of the epoxyoctadecanoate isomers. Known epoxyoctadecanoate isomers were used to establish this point.

Quantitation with Standards

Cleavage of collected cis- and trans-epoxyoctadecanoate isomers to aldehyde and alde-
GC METHOD FOR DOUBLE BOND LOCATION

TABLE I

Periodic Acid Cleavage of Epoxyoctadecanoate Standards

<table>
<thead>
<tr>
<th>Epoxyoctadecanoate</th>
<th>Cleavage fragment</th>
<th>Percent(^{a}) aldehyde</th>
<th>Percent aldehyde-ester(^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-6,7</td>
<td>12A</td>
<td>53.4</td>
<td>46.6</td>
</tr>
<tr>
<td></td>
<td>6AE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>trans-9,10</td>
<td>9A</td>
<td>53.3</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td>9AE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-9,10</td>
<td>9A</td>
<td>52.7</td>
<td>47.3</td>
</tr>
<tr>
<td></td>
<td>9AE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-12,13</td>
<td>6A</td>
<td>54.6</td>
<td>45.4</td>
</tr>
<tr>
<td></td>
<td>12AE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Percentages calculated from peak areas corrected for number of ionizable carbons.

\(^{b}\)A = aldehyde and AE = aldehyde-ester

hydro-esters was carried out on several known standards. The GC curves in Figure 1 are representative of HIO\(_4\) cleaved epoxidized octadecenoate standards. Both the HIO\(_4\)-Et\(_2\)O and HIO\(_4\)-CH\(_2\)Cl\(_2\) reagents were used to cleave the epoxyoctadecanoates and found to produce good results. However, the HIO\(_4\)-CH\(_2\)Cl\(_2\) reagent was preferred because it was easier to use. The HIO\(_4\)-Et\(_2\)O procedure (12) as modified and described in this article also produced lower yields of cleavage products and more extraneous peaks than the HIO\(_4\)-CH\(_2\)Cl\(_2\) procedure (11). Furthermore, the HIO\(_4\)-Et\(_2\)O procedure tended to overoxidize the aldehydes slightly to the corresponding acids. These acids were removed by sodium bicarbonate treatment. The HIO\(_4\)-CH\(_2\)Cl\(_2\) cleaved epoxides did not require this kind of treatment. Occasionally the HIO\(_4\)-Et\(_2\)O method produced an extraneous peak which had a retention time slightly less than the nonaldehyde peak. The problem of extraneous peaks was eliminated effectively by careful purification of the ether.

The GC analysis of a HIO\(_4\)-CH\(_2\)Cl\(_2\) cleaved cis-epoxyoctadecanoate mixture containing known amounts of 9, 12, and 15 positional octadecenoate isomers (as determined by capillary GC) is shown in Figure 2. The GC data from a HIO\(_4\) cleaved sample are first computer integrated and the peak areas corrected for the number of ionizable carbons. Percentages are calculated for each peak and the data printed out. The aldehyde-ester percentages are directly related to bond positions in the sample. The percentages listed in Table III and Table IV for commercial vegetable shortening and salad oil were calculated by this method and provide a major savings in time required for these calculations.

The GC curves in Figures 1 and 2 contain a small peak which has the same retention time as epoxyoctadecanoate. The size of this peak diminishes with time as the HIO\(_4\) cleavage reaction proceeds.

Quantitation of the acetaldehyde and propenal peaks are difficult because of interference by the solvent peaks. The data in Table I summarize the corrected peak areas (7,9,18,19) obtained from the cis- and trans-epoxyoctadecanoate standards shown in Figures 1 and 2.

The HIO\(_4\) cleavage data are compared to similar data obtained by reductive ozonolysis. Peak areas were integrated by using a computerized on-line data acquisition system and then converting the peak areas to area/ionizable carbon (7,9,18). Correction of peak areas is required because the FID does not respond equally to equimolar amounts of aldehyde and aldehyde-esters of varying chain lengths or mol wt. The correction is obtained by simply programing the computer to divide the total peak area by the appropriate number of ion-

TABLE II

Comparison of Methods for Double Bond Location in Octadecenoate Mixture

<table>
<thead>
<tr>
<th>Octadecenoate</th>
<th>Ozonolysis, (^{a}) %</th>
<th>Periodic acid, (^{a}) %</th>
<th>Capillary GC, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-9</td>
<td>18.9</td>
<td>18.9</td>
<td>18.5</td>
</tr>
<tr>
<td>cis-12</td>
<td>54.1</td>
<td>55.0</td>
<td>53.5</td>
</tr>
<tr>
<td>cis-15</td>
<td>27.0</td>
<td>26.2</td>
<td>28.0</td>
</tr>
</tbody>
</table>

\(^{a}\)Percentages calculated from peak areas corrected for number of ionizable carbons.
### TABLE III

Double Bond Distribution in Commercial Shortening

<table>
<thead>
<tr>
<th>Double bond position</th>
<th>trans- (HIO₄)ᵃ</th>
<th>cis- (HIO₄)ᵃ</th>
<th>Total (Cal)ᵇ</th>
<th>Total (O₃)ᶜ</th>
<th>trans- (O₃)ᵈ</th>
<th>cis- (O₃)ᵈ</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1.5</td>
<td>—</td>
<td>0.9</td>
<td>0.6</td>
<td>1.0</td>
<td>3.5</td>
</tr>
<tr>
<td>8</td>
<td>6.6</td>
<td>2.4</td>
<td>5.5</td>
<td>4.2</td>
<td>4.9</td>
<td>8.5</td>
</tr>
<tr>
<td>9</td>
<td>21.5</td>
<td>67.0</td>
<td>47.4</td>
<td>47.9</td>
<td>49.1</td>
<td>20.0</td>
</tr>
<tr>
<td>10</td>
<td>26.0</td>
<td>5.9</td>
<td>14.4</td>
<td>14.3</td>
<td>14.0</td>
<td>30.0</td>
</tr>
<tr>
<td>11</td>
<td>22.7</td>
<td>8.0</td>
<td>14.5</td>
<td>14.2</td>
<td>14.2</td>
<td>22.5</td>
</tr>
<tr>
<td>12</td>
<td>10.5</td>
<td>13.1</td>
<td>11.8</td>
<td>12.0</td>
<td>12.4</td>
<td>8.5</td>
</tr>
<tr>
<td>13</td>
<td>4.3</td>
<td>1.7</td>
<td>5.3</td>
<td>2.8</td>
<td>2.6</td>
<td>4.5</td>
</tr>
<tr>
<td>14</td>
<td>2.2</td>
<td>1.8</td>
<td>1.7</td>
<td>2.0</td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>15</td>
<td>3.8</td>
<td>—</td>
<td>0.5</td>
<td>1.6</td>
<td>0.7</td>
<td>—</td>
</tr>
</tbody>
</table>

ᵃOctadecenoate fraction was epoxidized and then cleaved with HIO₄.
ᵇPercentages calculated from trans-content (41.9%) of total octadecenoate fraction and percentage of double bond in each position as determined by HIO₄ cleavage.
ᶜTotal octadecenoate fraction was cleaved by reductive ozonolysis.
ᵈThese results were estimated from the bar graphs in reference 16.

TABLE III illustrates carbons in the compound. This correction is only a first approximation (7,8,17,18), but it does provide reasonable areas for a homologous series of compounds. The usual discrepancies which have been observed previously for aldehyde and aldehyde ester data were found.

Table II compares the epoxidation-HIO₄ cleavage method with capillary GC analysis and reductive ozonolysis. The agreement between capillary GC analysis, reductive ozonolysis, and HIO₄ cleavage data in Table II is good. The mixture used in Table II was chosen because it can be separated by capillary GC which provides a good check on the other two procedures which are both cleavage methods.

Figures 1 and 2 and Tables I and II establish the quantitative accuracy of using HIO₄ cleavage of epoxyoctadecanoates as a means for determining double bond distribution in octadecenoate samples.

### Commercially Hydrogenated Samples

The entire sequential procedure used in the all GC procedure was tested by determining the double bond distribution in cis- and trans-octadecenoate fractions isolated from commercially available salad oil and vegetable shortening. Results obtained by this procedure were compared with data compiled by a liquid chromatographic procedure (16). The all GC procedure used preparative GC to obtain a pure octadecenoate (monoene) fraction from the trans-esterified triglycerides. Small amounts (2-3%) of methyl stearate and methyl linoleate impurities occasionally were present in the monoene fraction, but these impurities do not interfere with subsequent reactions or separation, since they are separated readily after the sample has been epoxidized. The sample size required for the preparative GC step depends, of course, upon the octadecenoate content of the sample.

The data in Figure 3 and Table III were obtained from the analysis of a partially hydrogenated vegetable shortening. The GC curves in Figure 3 were obtained by HIO₄-Et₂O cleavage of epoxyoctadecanoates collected from a Packard GC equipped with a 10:1 splitter. Ca. 0.3-0.4 mg epoxyoctadecanoate is the maximum sample size which can be separated satisfactorily with a 4 mm x 300 cm 10% EGSS-X column. Larger samples cannot be separated completely, because they overload the column. Collection of 2-3 mg octadecanoate is usually more than sufficient sample for epoxidation and further analyses.

The data in Table IV are from a commercially available vegetable oil prepared by partial selective hydrogenation and winterization of soybean oil. The sample was analyzed by the all GC procedure. The GC analysis of the trans-fraction in Figure 3 shows the applicability of the method to samples containing a wide distribution of positional isomers. The peak following the methyl 16-formylhexadecanoate (C₁₆AE) peak is uncleaved epoxyoctadecanoate and will overlap partially the methyl 17-formyloctadecanoate peak.

The data in Table IV illustrate the analyses...
Double Bond Distribution in Commercial Salad Oil

<table>
<thead>
<tr>
<th>Double bond position</th>
<th>trans- (HIO₄)ᵃ</th>
<th>cis- (HIO₄)ᵇ</th>
<th>Total (HIO₄)ᶜ</th>
<th>Total (Cal)b</th>
<th>trans- (O₃)c</th>
<th>cis- (O₃)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>7.3</td>
<td>—</td>
<td>1.4</td>
<td>0.8</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>8.2</td>
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<td>2.1</td>
<td>3.3</td>
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<td>—</td>
</tr>
<tr>
<td>9</td>
<td>14.5</td>
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<td>58.2</td>
<td>59.5</td>
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<td>8.1</td>
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<td>—</td>
</tr>
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<td>11</td>
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<td>—</td>
</tr>
<tr>
<td>14</td>
<td>2.8</td>
<td>—</td>
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<td>0.7</td>
<td>2.0</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>1.0</td>
<td>—</td>
<td>0.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

ᵃOctadecenoate fraction was epoxidized and then cleaved with HIO₄.
ᵇPercentages calculated from trans-content (23.1%) of total octadecenoate fraction and percentage of double bond in each position as determined by HIO₄ cleavage.
ᶜThese results were estimated from the bar graphs of reference 16.

DISCUSSION

The HIO₄ cleavage of epoxidized octadecenoate isomers produced quantitative data which are as accurate as reductive ozonolysis data for determining double bond distribution in monoene fractions from partially hydrogenated vegetable oils. Ozonolysis is still the method of choice if only total double bond distribution of the monoene fraction is required. When double bond distribution data are needed for individual cis- and trans-monoene fractions (20), the epoxidation-HIO₄ cleavage procedure should be considered since the all GC procedure requires less total work and time. A comparison of the all GC procedure with an earlier method (16), both of which yield complete analytical information on geometric and positional octadecenoate isomers, is in order. Starting with the same methyl esters from hydrogenated samples, the earlier method (16) employed a rubber reverse-phase chromatographic column to separate the C₁₈ isologues, i.e. octadecanoates, octadecenoates, octadecadienoates, and octadecatrienoates. Subsequently, a silver macroreticular ion exchange resin column was used to separate the cis-octadecenoates from trans-octadecenoates. Double bond positions in the cis- and trans-fractions then were determined by microozonolysis pyrolysis (5). In the present all GC procedure, an EGSS-X GC column separates the isologues. After epoxidation of the octadecenoates, the cis- and trans-epoxides are separated by an EGSS-X column and the collected fractions are cleaved by HIO₄. The cleavage products (aldehyde-esters and aldehydes) are analyzed with an OV 17-225 GC column.

The results in Tables III and IV and Figure 3 are compared with analysis of samples A and E in Figures 4 and 5 in reference 16. Note that...

FIG. 3. Analysis of octadecenoate fraction from partially hydrogenated vegetable shortening (a) total octadecenoate sample, (b) trans-octadecenoate fraction and (c) cis-octadecenoate fraction. GC conditions are the same as used in Figure 1. Periodic acid cleaved.
both methods give a similar pattern of results, but there are fairly large variances between the values for some individual positional isomers. Since the individual values under the columns total (\(\text{HIO}_4\)), total (\(\text{Cal}\)), and total (\(\text{O}_3\)) in Table III agree quite well, we feel the \(\text{HIO}_4\) cleavage data are more accurate than the ozonolysis-pyrolysis results given in reference 16. However, this increased accuracy may be due to better GC instrumentation and integration methods, rather than experimental procedural or separation techniques. Also the values listed in Tables III and IV were estimated from bar graphs in reference 16 and may contain appreciable errors. In addition to being more accurate, the total system reduces the total sample requirement from ca. 4 g for the liquid chromatographic ozonization procedure (16) to ca. 10 mg or less, and the elapsed analysis time/sample from 3 days to 10 hr. This decrease in analysis time and sample size is possible, because separation of the monoene fraction by preparative GC requires only 30-40 min and 10 mg sample compared to ca. 8 hr and 4 g sample for a rubber column separation. Subsequent separation into cis- and trans-fractions requires ca. 1 hr by GC compared to 8 hr by silver resin column chromatography.

Both the \(\text{HIO}_4\)-Et<sub>2</sub>O and \(\text{HIO}_4\)-CH<sub>2</sub>Cl<sub>2</sub> methods give similar results; but the \(\text{HIO}_4\)-CH<sub>2</sub>Cl<sub>2</sub> method is simpler, more reliable, produces no extraneous peaks, and the yield of aldehyde and aldehyde-ester cleavage products is higher. The \(\text{HIO}_4\) cleavage and reductive ozonolysis procedures both require the FID response to be corrected (7,8). Correcting the peak areas by dividing peak area by the number of ionizable carbons results in relatively poor agreement between the corrected aldehyde and aldehyde-ester peak areas. The FID response must be calibrated with standards or known mixtures (18) to obtain correction factors which will provide good agreement between equimolar amounts of aldehydes and aldehyde-esters. Accurate detection of acetaldehyde and propionaldehyde is difficult because these peaks overlap the methylene chloride or ether solvent peak. This problem is not serious, since only the aldehyde-ester peak areas need to be used for double bond location in monoenes. Since each aldehyde-ester peak must have a corresponding aldehyde partner, the aldehyde peaks are used qualitatively here to aid in identifying aldehyde-ester peaks and for detecting extraneous peaks.

The epoxidation \(\text{HIO}_4\) cleavage sequence has been demonstrated for octadecenoate standards and for monoene fractions isolated from partially hydrogenated fats. The procedure should be applicable to any biological or synthetic octadecenoate sample which can be gas chromatographed.

ACKNOWLEDGMENT

Methyl octadecenoate standards and methyl esters of commercially hydrogenated vegetable oils were available from previous work by C.R. Scholfield of this laboratory.

REFERENCES


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