Gas Chromatographic Separation of Linoleic Acid Hydroperoxides as Trimethylsilyl Ethers of Methyl Hydroxystearates

ABSTRACT

The primary products, 9- and 13-hydroperoxy-octadecadienoic acids, from lipoxygenase catalyzed oxidation of linoleic acid, were converted into the trimethylsilyl ethers of methyl 9- and 13-hydroxystearates which were completely separated by gas chromatography on an OV-17 methyl silicone (50% phenyl groups), capillary column.

During the oxidation of linoleic acid, catalyzed by lipoxygenase, isomeric 13-hydroperoxy-9-cis, 11-trans-octadecadienoic acid and 9-hydroperoxy-10-trans, 12-cis-octadecadienoic acid are formed. Most workers convert these products into more stable compounds, often saturated methyl hydroxy- or ketoacid esters, before subsequent separation and identification work.

Methyl 9- and 13-hydroxystearates have been separated on a silica gel column (1) or by thin layer chromatography (TLC) (2). The column method needs more material than the TLC method which separated most of the methyl 2- to 18-hydroxystearates but had less resolution in the range of the 11- to 14-hydroxy isomers. By combined gas chromatography-mass spectrometry, the methyl 9- and 13-hydroxystearates were identified from their mixed mass spectra though no separation had occurred in the gas chromatography step (3). This method, however, has not been found suitable for quantitative work or routine analysis.

Trimethylsilyl (TMS) ethers of methyl hydroxy acids were easily prepared in good yields (6) and it was also possible to partially separate the TMS-ethers of methyl 12-hydroxystearate and methyl ricinoleate by gas chromatography (4,6). However, no reports have been found about the separation of the TMS-ethers of methyl 9- to 14-hydroxystearates which can be used in the analysis of hydroperoxides from oxidized fatty acids.

The main purpose of the present investigation was to enable a gas chromatographic separation of particularly the TMS-ethers of methyl 9- and 13-hydroxystearates because of the underlying need to analyze the hydroperoxides produced in linoleic acid oxidation. By analyzing a mixture of the 9- and 13-TMS derivatives in a combined gas chromatograph-mass spectrometer with different packed columns, both of the isomers appeared in a single peak as recorded by the ion current detector. Mass spectra were run several times during the elution, and by plotting the intensity of specific mass numbers against retention time, two overlapping peaks were obtained. In this way it was possible to study the tendency for the two isomers to separate on columns of different polarity. The stationary phase that gave the best results was used in a capillary column which separated the isomers completely.

Reference mass spectra were made with methyl 9-, 10-, 11-, 12-, 13- and 14-hydroxysearates. Mixed hydroperoxides obtained by oxidation of linoleic acid with soybean lipoygenase were converted into the corresponding methyl hydroxystearates (5) which were identified as the 9- and 13-hydroxyacid isomers.

LIPIDS, VOL. 6, NO. 2
methyl hydroxystearates and those obtained from the enzymatically produced hydroperoxides were converted into their TMS ethers (6). The resulting pyridine solutions, after centrifugation, were analyzed on a 67 m x 0.5 mm i.d. capillary column with a 0.8 μm film of OV-17, methyl silicone (50% phenyl groups), as the liquid phase in a Perkin-Elmer 900 gas chromatograph provided with a flame ionization detector. The inlet split ratio was 16:1, the gas flow through the column 9.5 ml He/min at room temperature; the temperature was 200°C, the injector temperature 270°C.

The gas chromatograms (Fig. 1a,b) of the TMS ethers of methyl 9- and 13-hydroxystearates and of the hydroxy acid isomers derived from the hydroperoxides produced during the oxidation of linoleic acid show that the 9- and 13-isomers were completely separated on the capillary column. In addition, they separated well from the methyl stearate which originated from the excess of linoleic acid in the enzyme incubation medium (Fig. 1b).

We also wanted to know if the column used in the above experiment was able to separate other trimethylsilylated isomers of methyl hydroxystearates within the 9- and 14-isomer range. From the gas chromatogram shown in Figure 1c it is obvious that the 9-, 12-, 13- and 14-TMS substituted isomers could be sufficiently separated for identification and quantitative analysis. Mixed 9- and 10-TMS and 9- and 11-TMS derivatives appeared as a shouldered peak and a double peak respectively (not shown). The absence of peaks around the separated isomers in Figure 1b then indicates that no significant amounts of hydroperoxides other than the 9- and 13-hydroperoxide isomers were produced in the enzymatic oxidation of linoleic acid and therefore the analysis by the present method confirms earlier findings (3,5,7).

The gas chromatographic procedure described above to separate the 9- and 13-TMS compounds has become a valuable tool in the analysis of hydroperoxides produced from unsaturated fatty acids. Unfortunately, other authors (3,5) have not commented on the overall yield of methyl hydroxystearates from hydroperoxides. Therefore, our work now in progress on the mass spectra of the TMS-ethers of methyl 8- to 14-hydroxystearates and a better separation of the 8-, 9-, 10- and 11-TMS isomers also includes quantitative analysis.

C.E. ERIKSSON
KARIN LEU
Section of Analytical Biochemistry, Swedish Institute for Food Preservation Research Fack, S-400 21 Göteborg 16, Sweden

FIG. 1. Gas chromatograms of TMS-ethers of methyl hydroxystearates: a and c; reference compounds; b; compounds derived from linoleic acid hydroperoxides. Peak 1, Methyl stearate. 2, TMS-ether of methyl-9-hydroxystearate. 3, TMS-ether of methyl-12-hydroxystearate. 4, TMS-ether of methyl-13-hydroxystearate. 5, TMS-ether of methyl-14-hydroxystearate.

in an LKB 9000 coupled gas chromatograph-mass spectrometer (3). By this procedure the remaining linoleic acid was converted into methyl stearate which separated from the mixed methyl hydroxystearates on the gas chromatographic column. Both the reference