Analysis of Hydroxy Fatty Acids as Pentafluorobenzyl Ester, Trimethylsilyl Ether Derivatives by Electron Ionization Gas Chromatography/Mass Spectrometry

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Electron ionization (EI) gas chromatography/mass spectrometry (GC/MS) analysis of pentafluorobenzyl ester–trimethyl silyl ether (PFB-TMS) derivatives of hydroxy-substituted fatty acids provides structural information comparable to that obtained in analysis of methyl ester–trimethyl silyl ether (Me-TMS) derivatives. Use of this derivative eliminates the need to prepare two separate derivatives, the PFB-TMS derivative for molecular weight determination by electron capture ionization (negative ions) analysis and the Me-TMS derivative for structural determination by EI GC/MS analysis. The relative abundance of fragment ions observed during EI GC/MS analysis of these derivatized unsaturated fatty acids indicates the location of the —OTMS substituents relative to double bond positions in those cases studied. The most abundant fragment ions are observed when the compound contains an unsaturation two carbon atoms removed from the —OTMS ether carbon (the β-OTMS position). The "saddle effect" observed in the GC/MS analyses of some derivatized monohydroxy unsaturated fatty acids is suggested to be due to a thermally allowed pericyclic double bond rearrangement and indicates the presence of a conjugated diene one carbon atom removed from the —OTMS ether carbon (the α-OTMS position). The saddle effect is most prominent for fatty acids that contain additional unsaturation separated by a single methylene unit from the conjugated diene moiety. *(J Am Soc Mass Spectrom 1995, 6, 40–51)*

Hydroxy unsaturated fatty acids are an important class of naturally occurring biological compounds enzymatically formed by specific lipoxygenases, by cytochrome P-450, or by autooxidation of unsaturated precursor fatty acids. Many of these compounds possess potent biological activity [1], and analysis of these lipids has been directed by requirements for sensitivity and complete structural information. The most sensitive method of detection is electron capture ionization (ECI) gas chromatography/mass spectrometry (GC/MS) analysis of the pentafluorobenzyl ester–trimethylsilyl ether (PFB-TMS) derivatives [2, 3]. This "soft ionization" technique allows detection of the M-PFB anion at the picogram level; however, because little or no fragmentation occurs, structure information regarding the location of hydroxy substituents is not obtained. Structure information has been obtained for such compounds when electron ionization (EI) GC/MS analysis of the methyl ester–trimethylsilyl ether derivative is employed [4].

In the present study, the PFB-TMS derivatives of hydroxy-substituted fatty acids have been evaluated for use in EI GC/MS analysis. These derivatives are prepared easily and quantitatively and it is advantageous to analyze a single derivative for both ECI and EI GC/MS analysis rather than two separate derivatives. Prior knowledge of retention time, obtained by ECI GC/MS where only carboxylate anions are observed, greatly facilitates identification of the compound of interest when analyzed in the EI mode. This is especially true for samples that have been obtained from complex biological matrices that may have closely eluting compounds even when capillary gas chromatography is employed.

In the present study fragmentation of the PFB-TMS–derivatized unsaturated fatty acids has been analyzed in terms of double bond positions relative to the trimethylsilyl ether positions. Analysis of the relative abundances of fragment ions allows prediction of double bond locations as well as hydroxy substituent positions in those cases studied. Also, the origin of the "saddle effect" [5, 6], or broadening of the signal

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observed in the GC/MS analysis of many unsaturated hydroxy fatty acids, has been studied and is attributed to thermal double bond rearrangement.

Methods

[6,7,14,15-d_4]-Leukotriene B_4 (LTB_4) was purchased from Biomol Research Laboratories (Plymouth, PA). Synthetic 5,12-dihydroxy-(6*Z*,8*E*,14*Z*)-eicosatrienoic acid (10,11-dihydro-LTB_4) was a generous gift from Dr. J. R. Falck (Southwestern Medical Center, University of Texas). All other hydroxy-substituted unsaturated fatty acids were obtained from Cayman Chemical (Ann Arbor, MI). Saturated hydroxy fatty acids were prepared by catalytic reduction of the standards. Fatty acids (1–5 μg) were dissolved in methanol (400 μL) that contained 5% Rh/Al_2O_3 catalyst (0.2–0.4 mg). Hydrogen was bubbled through the mixture for 2 min at room temperature, followed by centrifugation and removal of the methanol solution from the catalyst. The catalyst was washed with additional methanol and the combined methanol extracts were dried under nitrogen.

Pentafluorobenzyl ester (PFB) derivatives were prepared by treating the fatty acids (1–5 μg, dried under nitrogen) with a 10% solution (v/v) of *N*,*N*-diisopropylethylamine (Aldrich Chemical Co., Milwaukee, WI) in acetonitrile (50 μL) and a 10% solution (v/v) of pentafluorobenzyl bromide (Aldrich Chemical Co.) in acetonitrile (50 μL). Samples were kept at room temperature for 20 min, followed by evaporation under nitrogen. Trimethylsilyl ether derivatives were prepared by dissolving the PFB esters in acetonitrile (50 μL), followed by the addition of bis(trimethylsilyl)trifluoroacetamide (Supelco, Inc., Bellefonte, PA) (50 μL) or bis(trimethyl d_9-silyl)acetamide (MSD Isotopes, Montreal, Canada) (50 μL) and heating at 60 °C for 5 min. Samples were evaporated under nitrogen and redissolved in acetonitrile at 25–50 ng/μL for analysis by EI GC/MS or at 2–4 ng/μL for analysis by electron capture ionization ECI GC/MS.

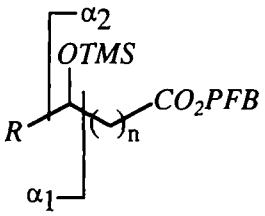
Derivatized fatty acids were analyzed by EI GC/MS via a Finnigan SSQ 70 (San Jose, CA) operating with an electron energy of 70 eV and a source temperature of 180 °C. ECI GC/MS analyses were obtained on the same system by using methane as the moderating gas and a source temperature of 150 °C. The mass spectrometer was interfaced to a Varian 3300 gas chromatograph (Walnut Creek, CA) with a 5-m × 0.25-mm DB-1, 0.25-μm film thickness, capillary column (J & W Scientific, Folsom, CA). For some analyses, a 10-m column was used. The injector temperature was maintained at 275 °C and the transfer line was maintained at 300 °C. Derivatized samples (1 μL) were injected into the gas chromatograph by using an initial column temperature of 150 °C followed by a 15 °C/min ramp to 310 °C. Full scans were acquired from *m/z* 50–700 at 1 scan/s.

TMS derivatized 12-hydroxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoic acid (12-HETE; 1 μg) and 15-hydroxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid (15-HETE; 1 μg) were evaporated under argon and sealed. The samples were heated at 200 °C for 20 min, cooled, and dissolved in the initial reverse phase (RP) high performance liquid chromatography (HPLC) mobile phase. RP HPLC analysis was performed on an Ultremex column (4.6- × 250-mm, 5-μM C-18; Phenomenex; Torrance, CA) with a mobile phase that consisted of methanol:H_2O, 0.05% acetic acid (pH adjusted to 5.8 with ammonium hydroxide) at an initial composition of 50% methanol followed by a linear gradient to 100% methanol in 50 min.

Results and Discussion

Monohydroxy Fatty Acids

Major fragment ions observed after EI GC/MS analysis of several PFB-TMS–derivatized monohydroxy fatty acids are listed in Table 1 and illustrated by the examples in Figure 1. Fragment ions were generally similar to those obtained in the analysis of methyl ester–TMS derivatives [4, 7]. However, even when significant M^+ ions were observed, no significant M-15 ion was present as expected for trimethylsilyl ether derivatives. The fragment ion at *m/z* 181 was observed for all derivatized hydroxy acids and is indicative of the PFB group. Additional fragmentations were dominated by bond scission adjacent to the —OTMS substituted carbon, designated α-OTMS fragmentation, and the observed ions contained the —OTMS substituted carbon. For PFB-TMS–derivatized saturated fatty acids, both α-OTMS fragmentations were abundant as demonstrated in Figure 1a for 12-hydroxy octadecanoic acid with observed ions at *m/z* 187 and 467. Because both α-OTMS fragment ions contained the —OTMS substituent, α-OTMS fragment ions were observed at 9 u higher when 12-hydroxy octadecanoic acid was analyzed as the PFB-d_9-TMS derivative with observed ions at *m/z* 196 and 476 (Figure 1b). The designations α_1 and α_2 in Table 1 refer to the fragmentations shown in Scheme I, where the fragment ion α_1 contains the trimethylsilyl ether group, but does not contain the PFB ester group (*m/z* 187 for PFB-TMS 12-hydroxy octadecanoic acid), whereas fragment ion α_2 contains both the trimethylsilyl ether group and the PFB ester group (*m/z* 467 for PFB-TMS 12-hydroxy octadecanoic acid).



Scheme 1.

Table 1. EI GC/MS of PFB-TMS derivatives of monohydroxy fatty acids

	Mass-to-charge ratio (abundance)			Trimethylsiloxy rearrangement	*m/z* 181
	M^+	α_1	α_2 2		
5-HETE	572 (*8*)	305 (*63*)	369 (*34*)	421 (*64*)	(*100*)
saturated	580 (< *1*)	313 (*53*)	369 (*100*)		(*15*)
8-HETE	572 (n d)[a]	**265** (*100*)[b]	409 (*2*)		(*27*)
saturated	580 (n d)	271 (*94*)	411 (*100*)		(*29*)
9-HETE	572 (< *1*)	253 (n d)	**421** (*100*)		(*40*)
saturated	580 (n d)	257 (*39*)	425 (*100*)		(*14*)
11-HETE	572 (< *1*)	**225** (*100*)	449 (n d)		(*38*)
saturated	580 (< *1*)	229 (*48*)	453 (*100*)		(*34*)
12-HETE	572 (n d)	213 (*4*)	**461** (*100*)		(*65*)
saturated	580 (n d)	215 (*57*)	467 (*100*)		(*24*)
15-HETE[c]	572 (*4*)	173 (*19*)	501 (*8*)	225 (*50*)	(*75*)
saturated	580 (< *1*)	173 (*100*)	509 (*60*)		(*20*)
13-HOTrE	546 (n d)	171 (n d)	**477** (*100*)		(*78*)
saturated	552 (n d)	173 (*100*)	481 (*35*)		(*37*)
13-HOTrE γ	546 (*7*)	173 (*11*)	475 (*27*)	225 (*100*)	(*60*)
			385 (*4*)		
saturated		*identical to 13-HOTrE*			
9-HODE	548 (*19*)	225 (*100*)	425 (*10*)	477 (*7*)	(*45*)
saturated	552 (n.d)	229 (*100*)	425 (*56*)		(*62*)
13-HODE	548 (*17*)	173 (*18*)	477 (*43*)	225 (*19*)	(*100*)
saturated		*identical to 13-HOTrE*			
12-HOME	550 (< *1*)	**187** (*100*)	465 (*5*)		(*17*)
saturated	552 (< *1*)	187 (*100*)	467 (*60*)		(*65*)
12-HHTrE	532 (*3*)	173 (*11*)	461 (*8*)	225 (*45*)	(*100*)
saturated	538 (< *1*)	173 (*100*)	467 (*67*)		(*35*)

[a] n d = not detected
[b] Underlined mass-to-charge ratio values in bold indicate fragmentation with loss of a neutral stabilized radical
[c] Base peak = *m/z* 73

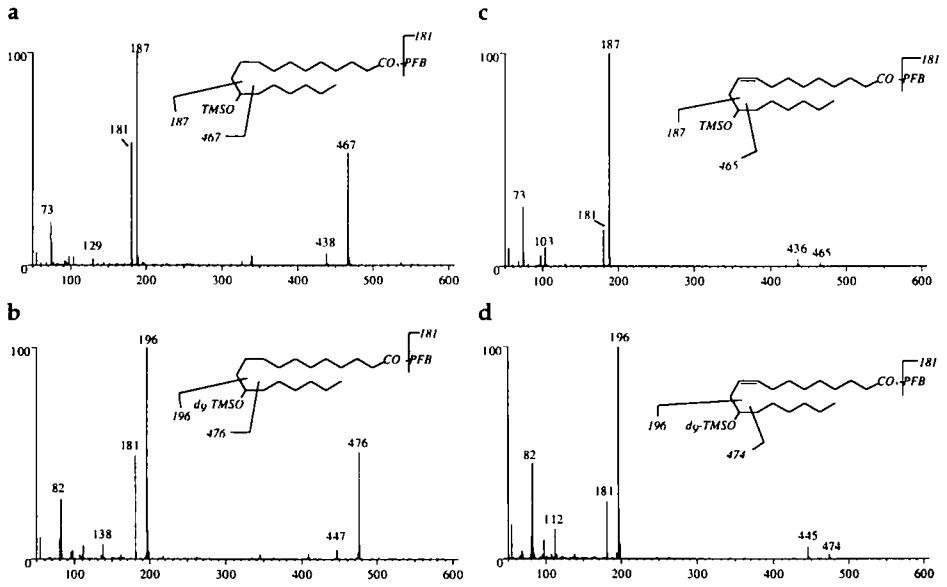
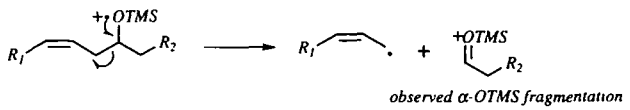


Figure 1. EI GC/MS spectra of (**a**) PFB-TMS-derivatized 12-hydroxy octadecanoic acid, (**b**) PFB-d_9-TMS-derivatized 12-hydroxy octadecanoic acid, (**c**) PFB-TMS-derivatized 12-hydroxy-9-octadec(mono)enoic acid, and (**d**) PFB-d_9-TMS-derivatized 12-hydroxy-9-octadec(mono)enoic acid.

The only other ions at high mass were of minor abundance and were observed at m/z 438 and 447 in Figure 1a and b, respectively. These correspond to fragmentation of the C11—C12 bond following migration of the TMS group to the carboxylate moiety. Rearrangement ions that involve migrations of TMS groups to carboxylic acid esters have been well documented [8, 9]. Similar low abundance ions also were observed at m/z 436 and 445 in Figure 1c and d, respectively, for the unsaturated analogs.

In contrast to the simple fragmentation of saturated monohydroxy fatty acid derivatives, introduction of a single double bond had a significant influence on EI-induced decomposition as shown in Figure 1c and d for derivatized 12-hydroxy-9-octadec(mono)enoic acid (12-HOME). For all PFB-TMS unsaturated monohydroxy fatty acids, the most abundant ions were observed when α-OTMS fragmentation occurred with loss of an allylic radical. The abundance of this ion is related to the loss of a neutral radical that may be stabilized by the adjacent double bond as shown in Scheme II. For derivatized 12-HOME, loss of a neutral stabilized radical resulted in an observed ion at m/z 187 (100% relative abundance), which shifted by 9 u to m/z 196 when 12-HOME was analyzed as the PFB-d_9-TMS derivative. The alternative α-OTMS bond scission that results in loss of the C-13 to C-18 neutral radical, which is not stabilized by double bond conjugation, was observed at only 5% relative abundance. Observed ions that result from loss of a neutral stabilized radical are underlined in Table 1 and were always the base peak in the mass spectrum of these compounds. In addition, when such an α-OTMS fragmentation was present, no molecular ion was observed or was observed at less than 1% relative abundance. The observed ion may contain the PFB ester group (α_2) as for 9-hydroxy-(5Z,7E,11Z,14Z)-eicosatetraenoic acid (9-HETE; m/z 421; Figure 2a), as well as for 12-HETE (m/z 461) and 13-hydroxy-(9Z,11E,15Z)-octadecatrienoic acid (13-HOTrE; m/z 477), or the PFB ester group may be lost as part of the stabilized radical moiety (α_1 fragmentation) as for 8-hydroxy-(5Z,9E,11Z,14Z)-eicosatetraenoic acid (8-HETE; m/z 265; Figure 2b) and 11-hydroxy-(5Z,8Z,12E,14Z)-eicosatetraenoic acid (11-HETE; m/z 225), as well as for 12-HOME (m/z 187).



Scheme II

When there is no unsaturation β, γ to the —OTMS-substituted carbon, α-OTMS fragmentation that results in loss of a stabilized neutral radical is not possible and significant $M^{\cdot+}$ ions were observed. Fragmentation at the α-OTMS position then occurred at a saturated site with formation of an nonstabilized neutral radical, by —OTMS rearrangement or at an apparent vinylic position. Significant α-OTMS fragmentation resulting in loss of a nonstabilized neutral radical occurred for 9-hydroxy-(10E,12Z)-octadecatrienoic acid (9-HODE, m/z 225) and for 13-hydroxy-(9Z,11E)-octadecatrienoic acid (13-HODE; m/z 477). Loss of a neutral nonstabilized radical also was observed for 5-hydroxy-(6E,8Z,11Z,14Z)-eicosatetraenoic acid (5-HETE; m/z 305), 15-HETE (m/z 501), 13-hydroxy-(6Z,9Z,11E)-octadecatrienoic acid (13-HOTrEγ; m/z 475), and for 12-hydroxy-(5Z,8E,10E)-heptadecatrienoic acid (12-HHTrE; m/z 461). However, for these compounds a more favorable α-OTMS fragmentation was observed that resulted from initial trimethylsiloxy rearrangement. Rearrangement of trimethylsiloxy was first proposed to account for the observed fragmentation in the EI spectrum of 12,17-dihydroxy-heptadecatrienoic acid derivatized as the methyl ester-TMS ethers [10]. As shown in Scheme III for 15-HETE, trimethylsiloxy rearrangement resulted in the loss of a neutral stabilized radical and the observed ion at m/z 225. The observed α-OTMS fragment ion following trimethylsiloxy rearrangement was observed at m/z 421 for 5-HETE, at m/z 225 for 13-HOTrE γ, and at m/z 225 for 12-HHTrE. Ions that resulted from trimethylsiloxy rearrangement also were

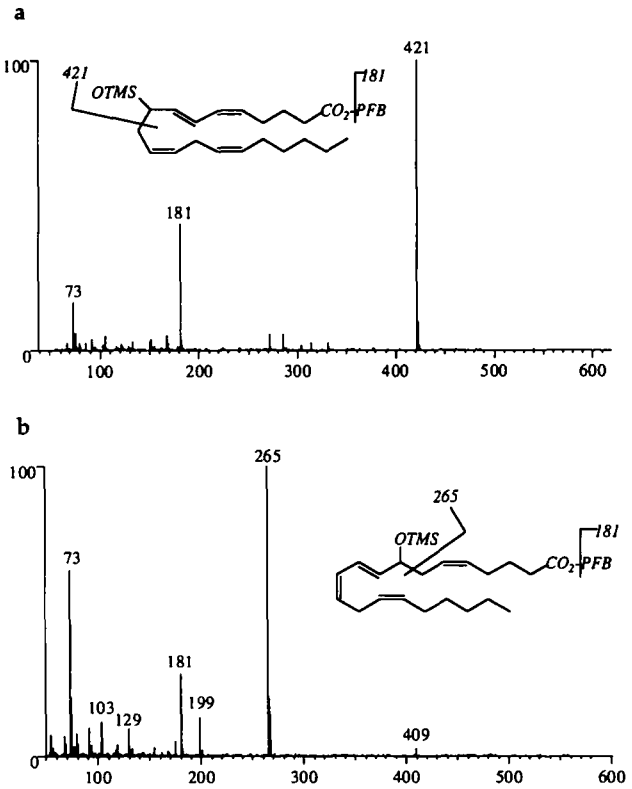
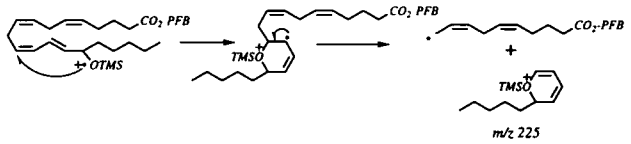


Figure 2. EI GC/MS analysis of the PFB-TMS derivatives of (**a**) 9-HETE and (**b**) 8-HETE.

observed for 9-HODE (m/z 477) and for 13-HODE (m/z 225), but for these compounds α-OTMS fragmentation that followed trimethylsiloxy rearrangement resulted in loss of a nonstabilized neutral radical and α-OTMS fragment ions formed with loss of nonstabilized neutral radicals from the nonrearranged compounds were more abundant.



Scheme III

Gas Chromatographic Saddle Effect Fragmentation at the α-OTMS position at an apparent vinylic position always was evidenced by a broadening of the ion current profile for that ion in GC/MS analysis. The total ion current profile then displayed the chromatographic broadening that has been termed the "saddle effect" [5]. The abundance of this type of ion was most prominent for compounds that contained a conjugated diene one carbon atom removed from the —OTMS carbon atom (the α-OTMS position) separated by a methylene group from additional unsaturation. The abundance of these fragment ions was increased by the use of longer GC columns, which required higher temperature for elution, suggesting a thermal double bond rearrangement. In fact, this may become the predominate species as shown in Figure 3 for PFB-TMS 15-HETE. With a 5-m GC column, PFB-TMS 15-HETE eluted with a narrow chromatographic signal at a column temperature of 224 °C (Figure 3a) and the predominate α-OTMS fragmentation resulted from trimethylsiloxy rearrangement with an observed ion at m/z 225. With a 10-m GC column, PFB-TMS 15-HETE eluted with chromatographic broadening around 277 °C and the fragment ions observed in the spectrum taken in the saddle suggested a rearranged compound with unsaturation at C-5,8,10,12 (Figure 3b). This rearrangement was suggested by the very abundant observed ion at m/z 173, which was the base peak, and the lack of a significant molecular ion. This pattern was consistent with α-OTMS fragmentation that results in loss of a neutral stabilized radical. This proposed thermal rearrangement of 15-HETE would result in a conjugated triene structure with a characteristic UV spectrum centered around a wavelength of 270 nm. This was substantiated by heating TMS-derivatized 15-HETE under argon at 200 °C for 20 min. Subsequent reverse phase (RP) HPLC analysis revealed three distinct products with triene chromophores observed at a wavelength of 270 nm (λ_{max} = 270 to 276 nm) as well as the unreacted initial 15-HETE that contained a conjugated diene chromophore observed at 230 nm (λ_{max}

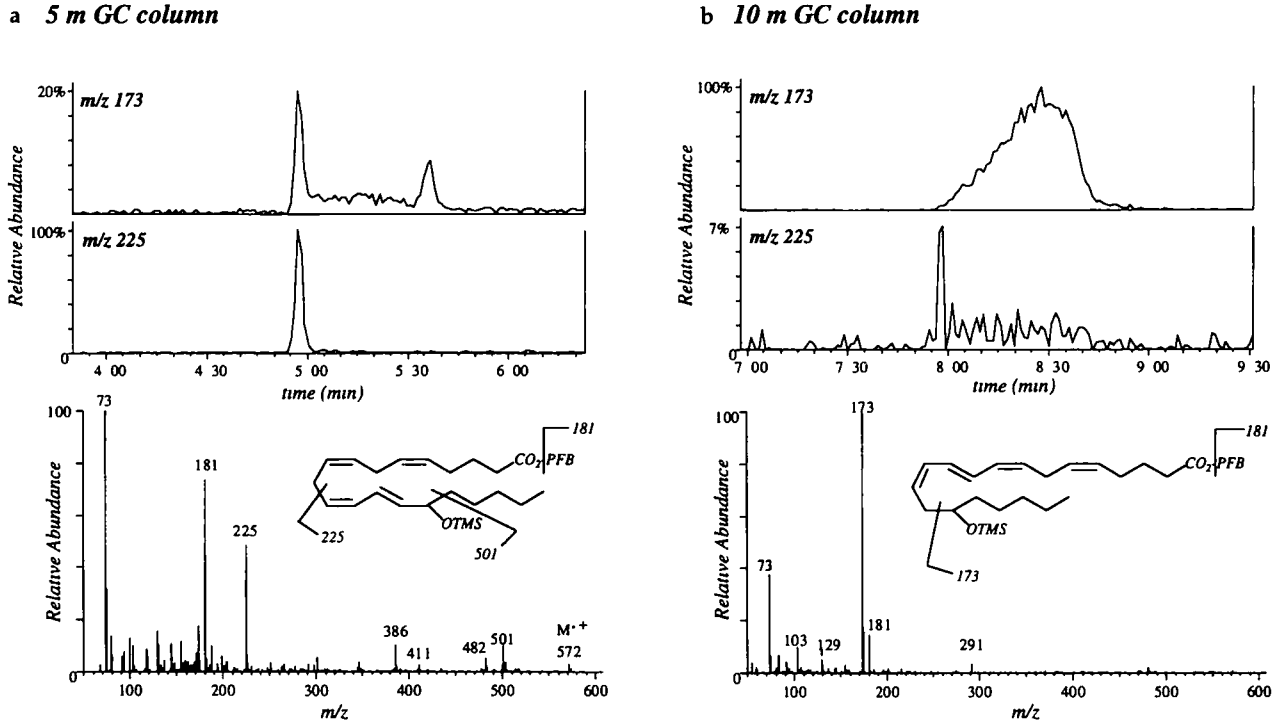


Figure 3. EI GC/MS analysis of the PFB-TMS derivative of 15-HETE. (a) Analysis obtained at low temperature GC conditions via a 5-m capillary column. Signal at retention time 4:57 min eluted at 224 °C with the spectrum shown. The signal at retention time 5.37 min (234 °C) was identical to that shown in (b). (b) Analysis obtained at high temperature GC conditions via a 10-m capillary column. The signal at retention time 8:28 eluted at 277 °C with the spectrum shown. The fragmentation pattern was consistent with the compound shown that resulted from double bond rearrangement prior to ionization

= 236 nm) (Figure 4). Following PFB-TMS derivatization, analysis of the collected trienes and remaining 15-HETE by electron capture ionization (ECI) GC/MS analysis (negative ions) via a 5-m GC column resulted in an observed carboxylate anion (M-181) at m/z 361 at the appropriate retention time for PFB-TMS 15-HETE as well as m/z 361 signals at retention times 20 and 40 s later. This suggested the compounds that elute in the saddle region contained conjugated triene structures.

GC/MS analysis of PFB-TMS–derivatized 12-HETE also resulted in chromatographic broadening with increasing GC column length with the two distinct EI spectra shown in Figure 5. Spectra taken in the saddle region were dominated by the observed ion at m/z 213, which suggests bond scission with loss of a stabilized neutral radical from the structure shown in Figure 5c. The spectrum taken at the beginning of the saddle region or when a 5-m GC column was used with lower elution temperature, was dominated by an observed ion at m/z 461, consistent with α_2 fragmentation and loss of a stabilized neutral radical from the nonrearranged compound (Figure 5d). Heat treatment of TMS-derivatized 12-HETE and RP HPLC analysis also revealed three distinct compounds with triene chromophores as well as unreacted 12-HETE similar to that shown for 15-HETE (Figure 4). ECI GC/MS analysis via a 5-m GC column of collected unreacted 12-

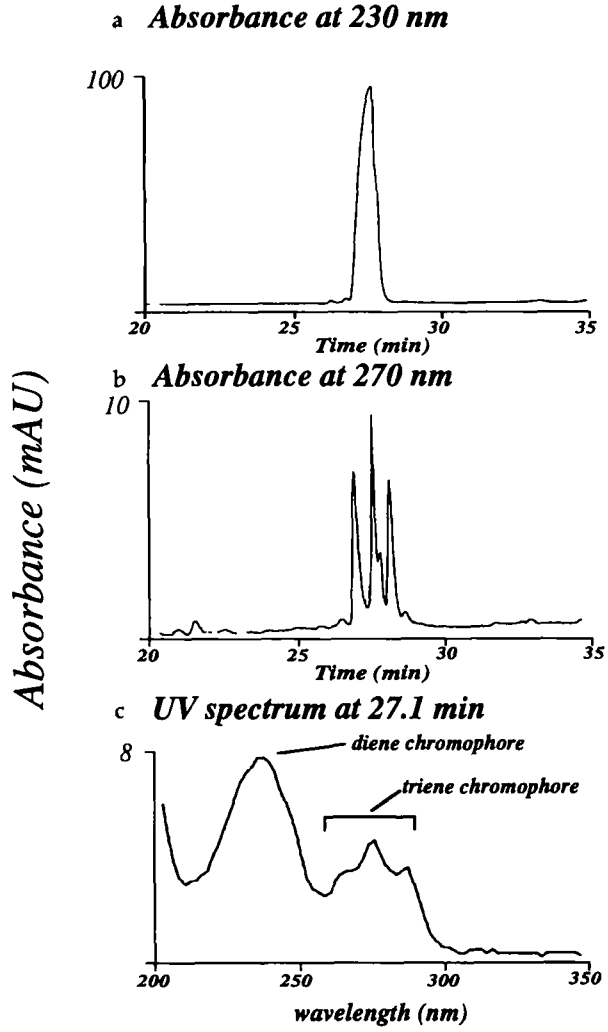


Figure 4. RP HPLC analysis of heat-treated 15-HETE with detection of (a) unreacted 15-HETE at 230 nm and (b) detection of 15-HETE following rearrangement to conjugated triene structures with detection at 270 nm. The UV spectra for the nonrearranged 15-HETE diene structure and for the earliest eluting rearranged compound that displayed a conjugated triene structure are shown in Figure 3c.

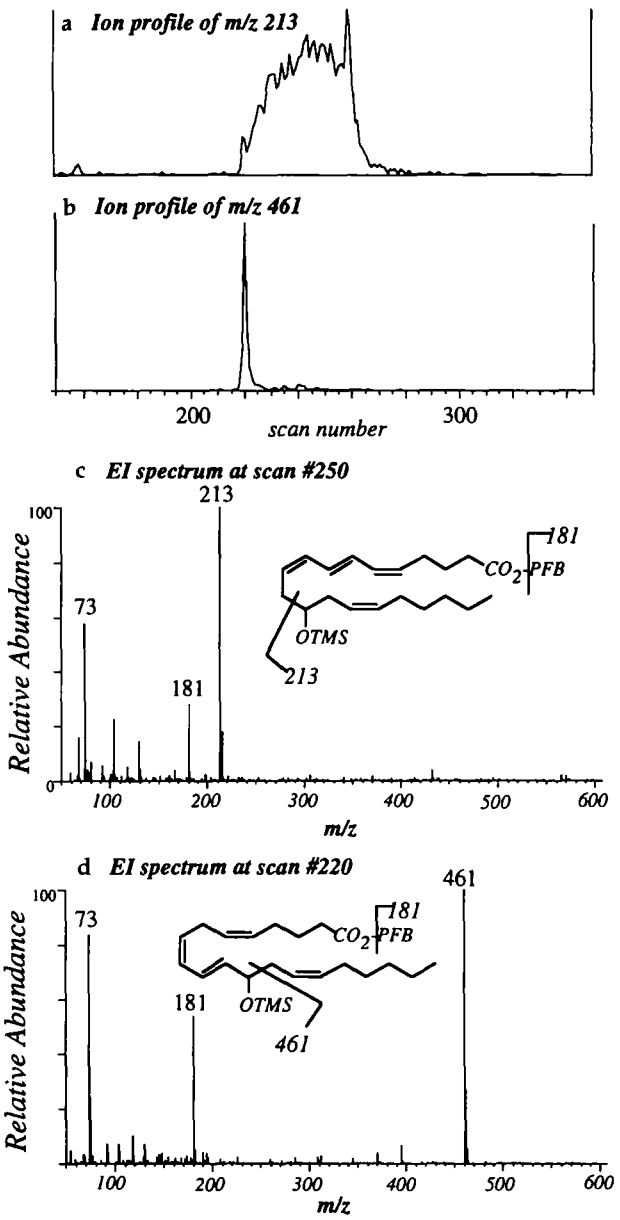
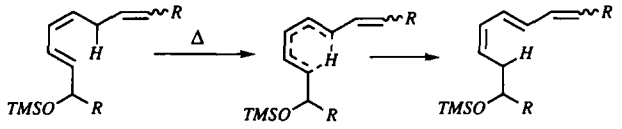


Figure 5. EI GC/MS analysis of the PFB-TMS derivative of 12-HETE on a 10-m GC capillary column showing (a) the saddle region that is due primarily to the ion current profile at m/z 213 and (b) the ion current profile at m/z 461 observed as a sharp profile before the saddle. (c) The EI spectrum in the saddle region and (d) the EI spectrum at the beginning of the saddle region.

HETE resulted in an observed carboxylate anion at m/z 361 at retention time 4:14 min (Figure 6a), and analysis of the collected trienes also resulted in observed carboxylate anions at m/z 361 with the earliest HPLC eluting triene observed at retention time 4:54 min (Figure 6b). The EI spectrum of the derivatized collected triene in Figure 6b was identical to that shown in Figure 5c.

These double bond rearrangements are consistent with a thermally allowed pericyclic reaction involving a 1,6 hydrogen transfer as shown in Scheme IV. The



Scheme IV

double bond stereochemistry at the α-OTMS and γ-OTMS position must be the trans-cis geometry. This was also suggested by the EI GC/MS behavior of PFB-TMS-derivatized 12-HHTrE, which also contains an α,γ-OTMS conjugated diene separated by a methylene group from an additional double bond; however, for 12-HHTrE the conjugated diene is trans-trans geometry (Figure 7). The EI GC/MS spectrum of PFB-TMS 12-HHTrE was identical at both low temperature conditions and at high temperature conditions and displayed no "saddle effect" and no significant fragmentation at the vinylic position. On-column thermal isomerization was also observed for 5-HETE (m/z 369), 8-HETE (m/z 409), and 13-HOTrE γ (m/z 173) as evidenced by broadening of the ion current profile for the respective apparent vinylic fragment ions for these compounds. These compounds all contain the trans-cis conjugated double bond geometry and also contain additional unsaturation separated by one methylene group from the conjugated diene. Analysis at high temperature GC column conditions then results in the formation of the more thermally stable conjugated triene structure. Apparent vinylic α-OTMS fragmentation was not observed for 9-HETE (m/z 253), 11-HETE (m/z 449), or 13-HOTrE (m/z 171) even at high temperature GC conditions. Although these compounds also contain the trans-cis conjugated diene structure, rearrangement would not result in a more thermally stable compound with increased conjugation because these compounds lack additional unsaturation one methylene unit beyond the conjugated diene. The ion current profile at m/z 425 for 9-HODE and at m/z 173 for 13-HODE, both apparent vinylic positions, also displayed broadening indicative of on-column isomerization of the trans-cis conjugated diene. However, these did not become the major fragment ions with increasing temperature conditions because isomerization also would not result in increased conjugation.

The thermal double bond rearrangement also may occur in the injector port and also may occur in the source as evidenced by the two distinct ion currents at m/z 173 for PFB-TMS 15-HETE shown in Figure 3a.

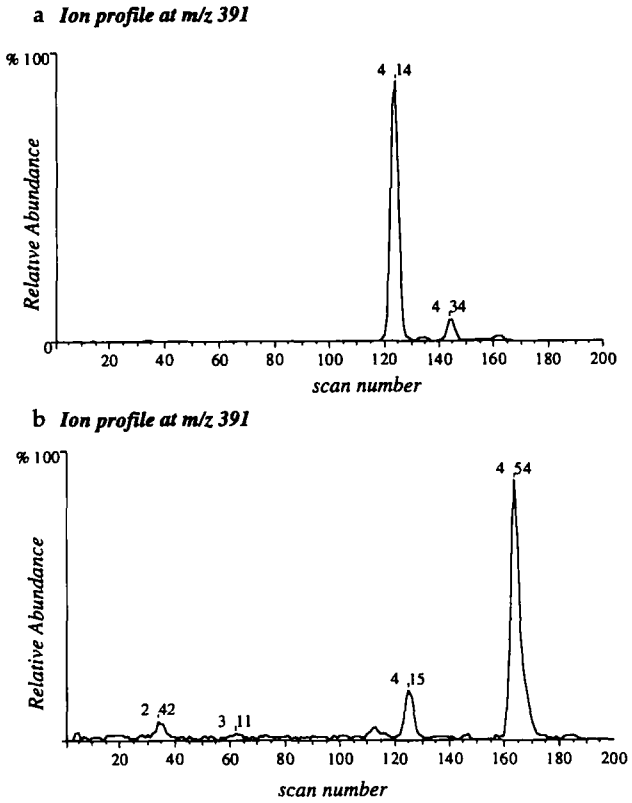


Figure 6. ECI (negative ions) GC/MS analysis that shows the ion profile for the carboxylate anion (m/z 391) of (a) PFB-TMS 12-HETE and (b) PFB-TMS 12-HETE as the rearranged compound that displayed a conjugated triene structure by UV analysis at 270 nm.

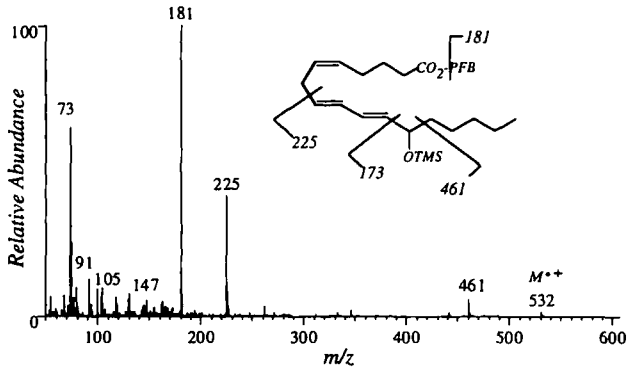


Figure 7. EI GC/MS analysis of the PFB-TMS derivative of 12-HHTrE.

However, rearrangement at these two sites that resulted in the apparent vinylic fragment ion was minor. The 1,6 hydrogen transfer depicted in Scheme IV also would imply a specific double bond geometry in the rearranged compound as well as in the starting compound and would suggest thermal rearrangement would result in the formation of a single triene of defined double bond geometry. The observation of three distinct trienes following heat treatment of 12- and 15-HETE may be due to cis-trans double bond isomerization subsequent to thermal double bond rearrangement.

Double Bond Location. EI GC/MS analysis of methyl ester–TMS ether derivatives of hydroxy-substituted fatty acids commonly is used with prior catalytic reduction of double bonds. This results in observation of both α-OTMS fragment ions and unequivocably establishes hydroxy substituent position. This is also true for PFB-TMS derivatives (Table 1). However, an understanding of the fragmentation of the unsaturated derivatives as outlined in the preceding text provides important information regarding double bond location when nonreduced compounds are analyzed. This is demonstrated in Figure 8 for the EI GC/MS analysis of PFB-TMS derivatives of 13-HOTrE, 13-HOTrE γ, and 13-HODE, which give identical spectra when analyzed as the reduced compounds. EI GC/MS analysis of the PFB-TMS–derivatized 13-HOTrE resulted in an observed base peak at m/z 477 (Figure 8). The abundance of this ion and the lack of a molecular ion confirm this fragmentation as α-OTMS fragmentation, which results in the loss of a stabilized neutral radical. Likewise, the observation of a significant molecular ion for 13-HOTrE γ and 13-HODE (Figure 8b and c) is consistent with the lack of β-OTMS unsaturation in these compounds. The most favorable α-OTMS fragmentation for 13-HOTrE γ resulted from initial trimethylsiloxy rearrangement with an observed fragment ion at m/z 225. This rearrangement and subsequent fragmentation resulted in the loss of a neutral stabilized radical and was more abundant than the fragmentation that resulted in an observed fragment ion at m/z 475 with loss of a nonstabilized neutral radical. For 13-HODE, trimethylsiloxy rearrangement followed by α-OTMS fragmentation also was observed (m/z 225), but this ion is formed with loss of a nonstabilized neutral radical. The observed ion that results from rearrangement is then less abundant than α-OTMS fragmentation that results in the observed ion at m/z 477. In addition, only derivatized 13-HOTrE γ displayed significant thermal on-column rearrangement with m/z 173 observed as the most abundant fragment ion when analyzed at high temperature column conditions.

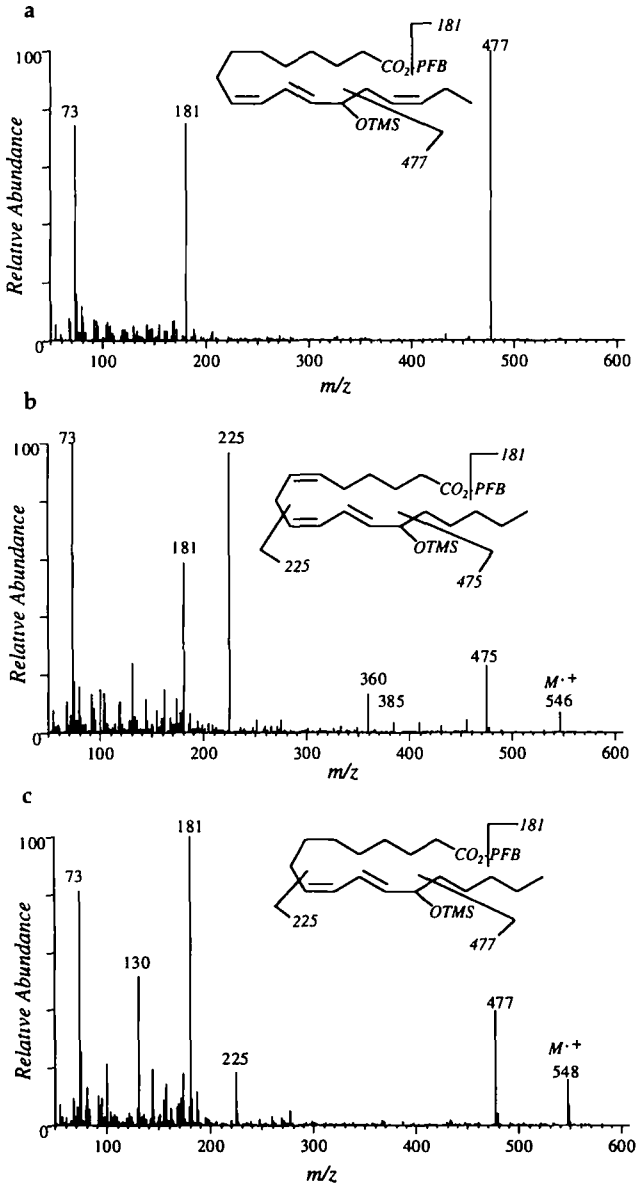
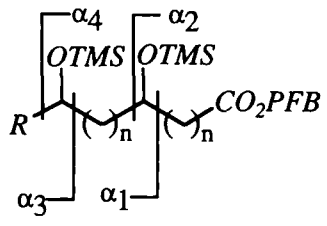


Figure 8. EI GC/MS analysis of the PFB-TMS derivatives of (**a**) 13-HOTrE, (**b**) 13-HOTrE γ, and (**c**) 13-HODE.

Dihydroxy Fatty Acids

Major fragment ions observed during EI GC/MS analysis of PFB-TMS–derivatized dihydroxy fatty acids are listed in Table 2 with the designations for fragment ions depicted in Scheme V. Fragment ions designated α_1 and α_3 contain the methyl terminus and fragment ions α_2 and α_4 contain the PFB ester terminus. Decomposition of derivatized dihydroxy compounds during electron ionization also was directed by the —OTMS position: α-OTMS fragmentation resulted in loss of a stabilized neutral radical predominating as for monohydroxy compounds; however, these ions often showed additional losses of TMSOH (loss of 90 u), which commonly was not observed for monohydroxy derivatives. These fragment ions are underlined in Table 2.



Scheme V

EI GC/MS analysis of 5,6-dihydroxy-(7*E*,9*E*,11*E*,14*Z*)-eicosatetraenoic acid (5,6-DiHETE) resulted in a base peak at m/z 369 (α_2), consistent with the

loss of a neutral, stabilized radical. A minor fragmentation (α_3) also was observed, which resulted in an observed ion at m/z 291 following loss of a nonstabilized neutral radical. Apparent vinylic fragmentation (α_4) was not observed.

Decomposition of PFB-TMS–derivatized 5,12-dihydroxy-(6*Z*,8*E*,10*E*,14*Z*)-eicosatetraenoic acid (LTB_4) was dominated also by loss of a neutral, stabilized radical (α_4) with observed ions at m/z 549, 459 (549-TMSOH) and 369 (549 − 2TMSOH) (Figure 9a). The second loss of TMSOH from m/z 549 must involve loss of a proton at C-6 or C-7 as evidenced by an observed ion at m/z 370 for [6,7,14,15-$^2H_4$]-5,12-dihydroxy-(6*Z*,8*E*,10*E*,14*Z*)-eicosatetraenoic acid (d_4-LTB_4). The ion observed at m/z 369 also was due in part to α_2 fragmentation, an apparent vinylic position. This was verified by the use of d_9-TMS–derivatized LTB_4, where both m/z 378 (α_2 fragmentation) and 369 (α_4 − 2[d_9-TMSOH]) were observed. The GC ion current profile for the apparent vinylic fragmentation that resulted in the observed ion at m/z 369 did not display broadening, which suggests that the double bond rearrangement that led to this fragmentation occurred in the source following ionization and not on the GC column. Double bond rearrangement of the ionized compound is also the likely mechanism that leads to the formation of the observed ion at m/z 395 (m/z 404 for PFB LTB_4 derivatized with d_9-TMS), which is formally a fragmentation of the C-7,8 bond. This fragmentation is substantiated by the observed

Table 2. EI GC/MS of PFB-TMS derivatives of dihydroxy fatty acids

	Mass-to-charge ratio (abundance)					
	M^+	α_1	α_2	α_3	α_4	m/z 181
5,6 DiHETE	660 (<1)	393 (n d)	**369** (100)[a]	291 (6)	471 (n d)	(30)
saturated	668 (n d)	401 (n d)	369 (67)	299 (100)	471 (n d)	(53)
LTB_4	550 (<1)	393 (n d)	369 (10)[b]	213 (n d)	**549** (17) 459 (8)	(52)[c]
saturated	668 (<1)	401 (4) 311 (10)	369 (54)	215 (86)	555 (19) 465 (10)	(100)
LTB_4 (d_9-TMS)	678 (1)	411 (n d)	378 (18)	222 (n.d)	**567** (27) 468 (18) 369 (5)	(40)[d]
d_4-LTB_4	664 (<1)	397 (6)[e]	369 (8)	215 (n.d)	**551** (26) 461 (27) 370 (11)	(97)[f]
10,11-Dihydro-LTB_4	662 (<1) 572 (2) 482 (4)	395 (15)[g] 305 (20)	369 (7)	213 (19)	**551** (4) 461 (9) 371 (6)	(79)[f]
8,15-DiHETE	660 (<1)	**353** (49) 263 (21)	409 (8)	173 (100)	589 (<1)	(29)
saturated	668 (<1)	359 (25)	411 (79)	173 (100)	597 (12)	(51)
8,15-DiHETE (d_9-TMS)	678 (n d)	**371** (44) 272 (17)	418 (14)	182 (100)	607 (n d)	(17)
5,6-DiHETrE	662 (4)	395 (n d)	369 (90)	293 (8) 203 (26)	**471** (26) 381 (93)	(96)[f]
saturated	668 (n.d.)	401 (n d)	369 (65)	299 (100)	471 (n d)	(34)
8,9-DiHETrE[h]	662 (2)	**355** (13) 265 (34)	409 (42)	253 (16)	**511** (6) 421 (100)	(50)
saturated	668 (n d)	359 (n d)	411 (65)	257 (100)	513 (n d)	(48)
11,12-HiHETrE[i]	662 (<1)	**315** (48) 225 (26)	449 (9)	213 (41)	**551** (3) 461 (42)	(60)
saturated	668 (n d)	317 (n d)	453 (36)	215 (100)	555 (n d)	(47)
14,15-DiHETrE[j]	662 (2)	**275** (97)	489 (4) 399 (10)	173 (100)	591 (n d)	(37)
saturated	668 (n d.)	275 (n d)	495 (54)	173 (100)	597 (n d)	(45)

[a] Underlined mass-to-charge ratio values in bold indicate fragmentation with loss of a neutral stabilized radical
[b] Contains contributions from α_2 fragmentation and from α_4-2(TMSOH)
[c] Base peak at m/z 73 Additional ion observed at m/z 395 (8) from C-7,8 fragmentation
[d] Base peak at m/z 82
[e] Ion at m/z 397 is due to rearrangement ion that involves fragmentation of C-7,8, analogous to m/z 395 in LTB_4 rather than formal α_1 fragmentation (see text)
[f] Base peak at m/z 73
[g] Ion at m/z 395 is due to C-7,8 fragmentation and ion at m/z 305 is due to α_1 fragmentation with loss of TMSOH (see text)
[h] 8,9-dihydroxy-(5*Z*, 11*Z*, 14*Z*)-eicosatrienoic acid
[i] 11,12-dihydroxy-(5*Z*, 8*Z*, 14*Z*)-eicosatrienoic acid
[j] 14,15-dihydroxy-(5*Z*, 8*Z*, 11*Z*)-eicosatrienoic acid

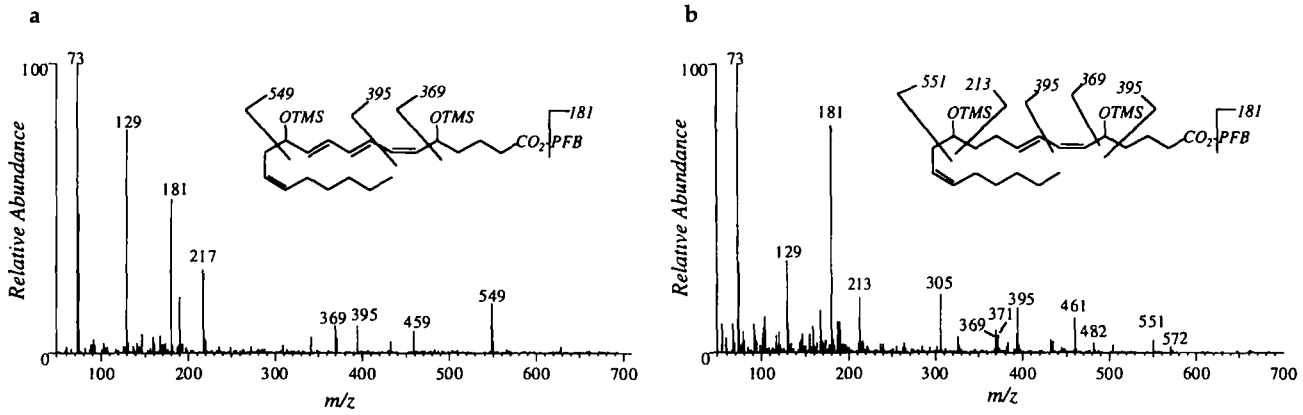


Figure 9. EI GC/MS analysis of the PFB-TMS derivatives of (a) LTB_4 and (b) 10,11-dihydro-LTB_4

shift to m/z 397 in the analysis of d_4-LTB_4 (6,7,14,15-$^2H_4$-LTB_4) and is observed at m/z 406 in the analysis of PFB d_4-LTB_4 derivatized with d_9-TMS. Fragment ions at m/z 129 and 217 were observed previously in the EI GC/MS analysis of the methyl ester–TMS derivative of LTB_4 [11] and are characteristic of TMS-derivatized polyhydroxy compounds. These ions were observed at m/z 138 and 235 when d_9-TMS derivatization was used, which confirmed the presence of one and two TMS moieties, respectively.

A major fragment ion that distinguishes 10,11-dihydro-LTB_4 from LTB_4 resulted from α_3 fragmentation with an observed ion at m/z 213 (Figure 9b). For LTB_4, this position is a vinylic position and bond scission at this site was not observed. For 10,11-dihydro-LTB_4, α_3 fragmentation occurs at a saturated position, but the neutral radical loss may be stabilized by rearrangement with the conjugated double bonds at C-7 and C-9 to form a stabilized cyclohexenyl radical. A common pathway for 10,11-dihydro-LTB_4 and LTB_4 decomposition was α_4 fragmentation, which resulted in loss of a neutral stabilized radical. This fragmentation was evidenced by observed ions in the spectrum of PFB-TMS 10,11-dihydro-LTB_4 at m/z 551, 461 (m/z 551 − TMSOH) and 371 (m/z 551 − 2TMSOH). Fragmentation at the α_2 position also occurred for 10,11-dihydro-LTB_4 as well as for LTB_4 with an observed ion at m/z 369. Another ion common to both spectra was observed at m/z 395 from fragmentation of the C-7,8 bond. This ion (m/z 395) also could arise from α_1 fragmentation of the C-4,5 bond in 10,11-dihydro-LTB_4 and would contain both OTMS substituents. However, in the spectrum of d_9-TMS-derivatized 10,11-dihydro-LTB_4, this ion was shifted to m/z 404, substantiating the presence of only one OTMS group. The fragment ion that resulted from α_1 fragmentation, which would be observed at m/z 413 for d_9-TMS-derivatized 10,11-dihydro-LTB_4, was present at less than 1% relative abundance. However, the occurrence of α_1 fragmentation at the C-4,5 bond was supported by the observed ion at m/z 305. This ion could arise from either C-7,8 fragmentation (m/z 395) with loss of TMSOH and would not contain an OTMS group or it could arise from α_1 fragmentation of the C-4,5 bond (m/z 395) with loss of one TMSOH group and would contain the second OTMS substituent. This ion was shifted to m/z 314 in the analysis of d_9-TMS-derivatized 10,11-dihydro-LTB_4 and suggested a facile TMSOH loss during α_1 fragmentation. This ion was not observed in the spectrum of LTB_4.

The PFB-TMS derivative of 8,15-dihydroxy-(5*Z*,9*E*,11*Z*,13*E*)-eicosatetraenoic acid (8,15-DiHETE) was the only DiHETE studied that showed significant on-column thermal rearrangement and was evidenced by a "reverse saddle" effect where the rearranged compound eluted before the nonrearranged compound (Figure 10a). The earlier GC retention time of the rearranged compound and the observation of only ions at m/z 409 and 173 were consistent with the structure shown in Figure 9a, where both ions arise from α-OTMS fragmentation with loss of a neutral, stabilized radical. This type of rearrangement has been observed previously in the EI GC/MS analysis of the methyl ester of 5,12-dihydroxy-(6*E*,8*Z*,10*E*,14*Z*)-eicosatetraenoic acid and requires the specific trans-cis-trans double bond stereochemistry of the conjugated triene between the hydroxy substituents [6]. Thermal rearrangement of the PFB-TMS derivative of 8,15-DiHETE also was minimized by use of a shorter GC column (Figure 10b). For the nonrearranged compound, significant α_1 fragmentation was observed, which results from loss of a stabilized neutral radical. Surprisingly, the base peak was due to α_3 fragmentation (m/z 173), which is an apparent vinylic fragmentation. This fragmentation, rather than α_1 − 2TMSOH, was verified by use of the d_9-TMS derivative where the α_3 fragmentation resulted in an observed ion at m/z 182. This ion must arise from rearrangement of the ionized compound (source rearrangement) when the initial site of ionization is at the C-15 —OTMS position. Apparently, source rearrangement of the ionized compound to the structure shown in Figure 9a is a faster process than loss of a nonstabilized neutral radical (α_4, m/z 589).

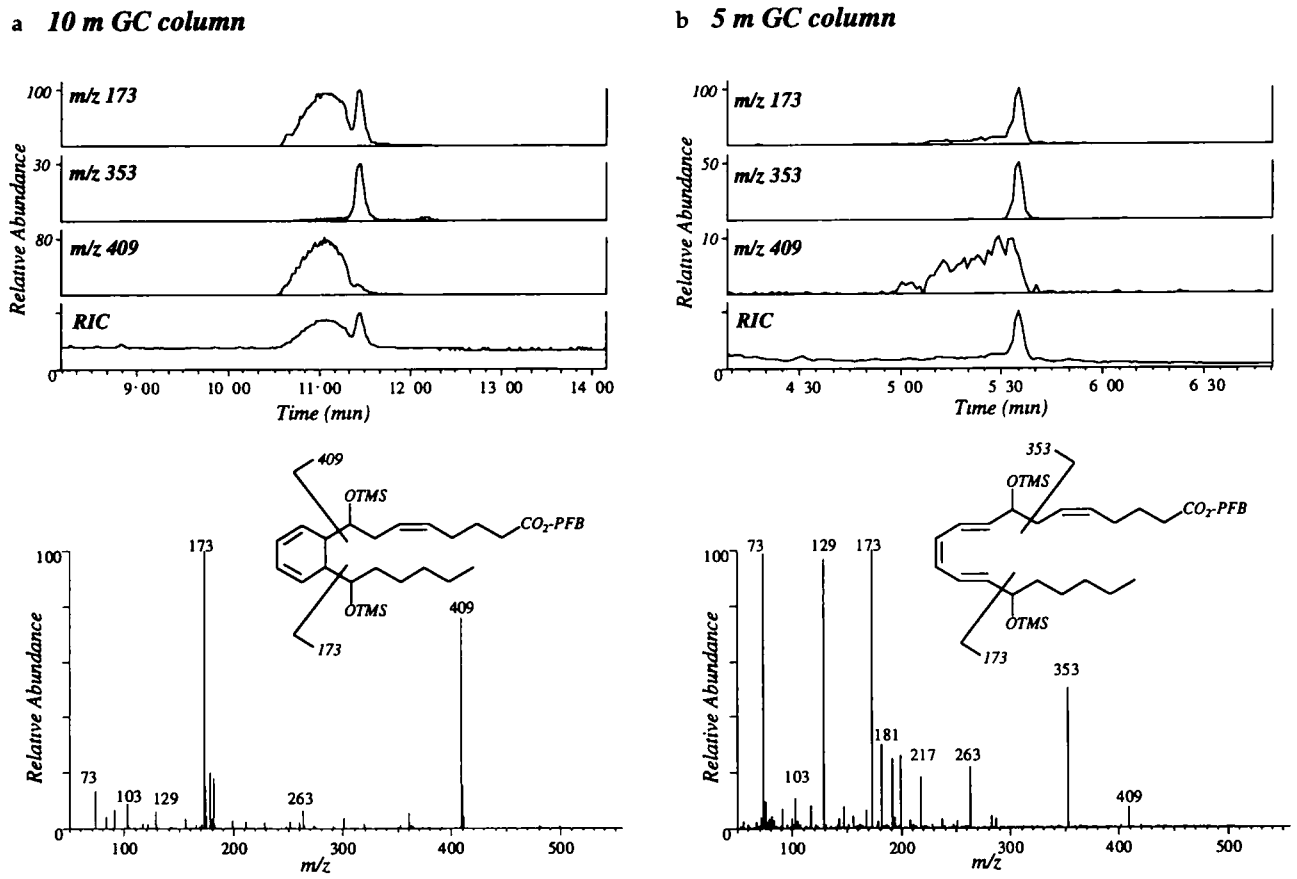


Figure 10. EI GC/MS analysis of the PFB-TMS derivative of 8,15-diHETE. (a) Analysis obtained at high temperature GC conditions via a 10-m capillary column. Signal at retention time 11 04 min eluted at 304 °C with the spectrum shown. Fragment ions are consistent with the rearranged structure shown (b) Analysis obtained via a 5-m capillary column The spectrum for the compound eluting at 5 35 min at 234 °C is shown

The remaining compounds in Table 2 are diols that all showed abundant fragment ions due to loss of a stabilized neutral radical. Additional fragmentation was observed between adjacent —OTMS groups that would involve loss of a nonstabilized neutral radical. However, this radical may be stabilized inductively by the trimethylsiloxy substituent.

The 5,12-dihydroxy compounds and 8,15-DiHETE showed all four possible α-OTMS fragmentations when analyzed as the saturated compounds. The diols, 5,6-DiHETE and the di-HETrE compounds, showed only the two fragment ions that result from α-OTMS fragmentation between the diols. For these saturated diol derivatives, the most abundant fragment ion did not contain the PFB ester group (α_3).

Conclusion

EI GC/MS analysis of monohydroxy-substituted unsaturated fatty acids derivatized as PFB ester–TMS ether compounds results in observed decomposition ions comparable to those observed in the analysis of the methyl ester–TMS ether derivatives with fragmentation directed by the charged OTMS group. The relative abundance of observed ions is related to the relative stability of the neutral radical that is lost during fragmentation If the compound contains a double bond at the β-OTMS position, bond scission occurs to give loss of a neutral stabilized radical (allylic radical). The resulting ion is the base peak in the spectrum and no molecular ion is observed or is present at less than 1% relative abundance. If there is no β-OTMS unsaturation, a molecular ion is observed. Fragmentation results in loss of a nonstabilized radical or fragmentation occurs following —OTMS rearrangement. If fragmentation following —OTMS rearrangement results in loss of a stabilized radical, the resulting ion is more abundant than the ion that results from loss of a nonstabilized radical from the nonrearranged compound.

Apparent vinylic bond scission of the PFB-TMS derivatives of monohydroxy unsaturated fatty acids is primarily due to on-column thermal rearrangement and is evidenced by broadening in the ion current profile for that ion. This occurs for methyl ester–TMS ether derivatives as well as for PFB-TMS derivatives,

but may be more pronounced for the PFB-TMS derivatives for which retention times are increased by 2.5 equivalent carbon units, on average, as compared to the methyl ester derivatives. This may be minimized by the use of a shorter GC column and lower elution temperatures [12]. Thermal rearrangement occurs for compounds that contain a trans-cis conjugated diene at the α-OTMS position. This rearrangement is consistent with a thermally allowed pericyclic reaction that involves a 1,6 hydrogen transfer and is most pronounced for those compounds that contain additional unsaturation separated from the diene system by a methylene unit. For these compounds, rearrangement results in a more thermally stable extended conjugated system.

Decomposition ions observed in EI GC/MS analysis of PFB-TMS–derivatized dihydroxy unsaturated fatty acids also are comparable to those observed in analysis of the methyl ester–TMS ether derivatives. Fragment ions formed with loss of a neutral stabilized radical are favored as for the monohydroxy compounds, but these may be complicated by additional losses of TMSOH.

Acknowledgment

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