Double Bond Migration to Methylidene Positions During Electron Ionization Mass Spectrometry of Branched Monounsaturated Fatty Acid Derivatives

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Electron ionization mass spectra of several monounsaturated methyl-branched fatty acid methyl and trimethylsilyl esters were examined. These spectra exhibited some intensive fragment ions, whose formation could be explained after double-bond migration to methylidene position. This preferential migration (substantiated by deuterium labeling) acts significantly in the case of monounsaturated fatty acid methyl and trimethylsilyl esters possessing a methyl branch localized between the penultimate and the C_4 positions (relative to the ester group), whatever the position of the double-bond. Allylic cleavage and γ-hydrogen rearrangement of the ionized methylidene group thus formed afforded very interesting fragment ions, which could be particularly useful to determine branching positions of monounsaturated methyl-branched fatty acid methyl and trimethylsilyl esters without additional treatment. (J Am Soc Mass Spectrom 2009, 20, 1997–2005) © 2009 American Society for Mass Spectrometry

Gas chromatography/electron ionization mass spectrometry of methyl (for a review see [1]) and trimethylsilyl [2] esters constitutes a particularly powerful technique for the identification of fatty acids. Unfortunately, the mass spectra of methyl and trimethylsilyl esters of monoenoic fatty acids have no information that helps to locate the position of double bonds. While there have been suggestions that such information can be obtained from close examination of certain minor peaks in the spectrum, the value of such techniques seems doubtful. There is no feature that permits location of the double-bond, because this can migrate to any position when the alkyl chain is ionized in the mass spectrometer. To get around the problem of location of double bonds, it is possible to prepare specific derivatives of unsaturated fatty acids that 'fix' the double-bond. Very many have been described. The more commonly employed are dimethyldisulfide adducts (which have excellent mass spectrometric properties and are prepared in a simple one-pot reaction) [3, 4] and vicinal trimethylsilyl ethers arising from stereospecific OsO_4 oxidation of double bonds [5]. Alternatively, picolinyl esters [6, 7] or DMOX [8] or pyrrolidine [9] derivatives can be utilized to locate double bonds. In these last cases, the carboxyl group is derivatized with a reagent containing a nitrogen atom. When the molecule is ionized in the mass spectrometer, the nitrogen atom, not the alkyl chain, carries the charge, and double-bond migration is minimized.

Methyl-branched monounsaturated fatty acids have been detected in several bacteria [10–14]; they are also present in some fish [15] and sponge [16, 17]. Careful examination of EI mass spectra of methyl and trimethylsilyl derivatives of these compounds suggested to us that the presence of branching strongly favors the migration of the double-bond to the methylidene position. Such a "specific" migration, which could lead to misinterpretation of mass spectra of these compounds, would be, in contrast, very useful to indicate the position of branching on their alkyl chain without additional treatment. In the present work, we thus: (1) examined EI mass spectra of numerous methyl-branched monounsaturated fatty acids formally identified, and (2) carried out deuterium labeling to try to confirm this assumption.

Experimental

Fatty Acids

C_{15}-C_{18} iso- and anteiso-methyl-branched monounsaturated fatty acids, 11-methyloctadec-12-enoic and

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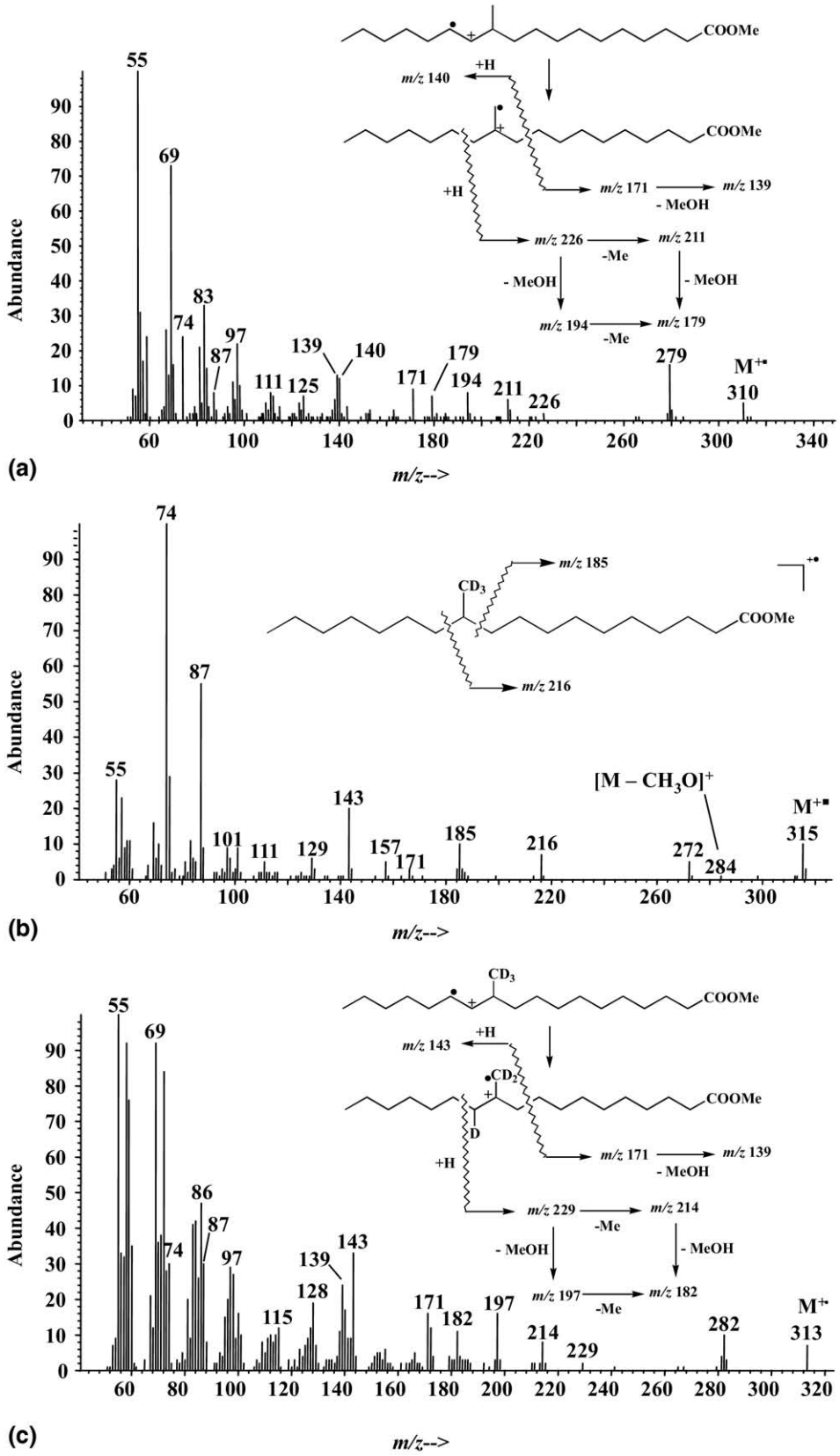


Figure 1. EI mass spectra of (**a**) 11-methyloctadec-12-enoic, (**b**) 11(D_3)-methyloctadecanoic, and (**c**) 11(D_3)-methyloctadec-12-enoic acid methyl esters.

12-methyloctadec-11-enoic acids, were obtained from bacterial lipid extracts [12, 14].

Deuterium Labeling

It was recently demonstrated that the formation of 11-methyloctadec-12-enoic acid in bacteria resulted from the methionine-mediated methylation of *cis*-vaccenic acid [14]. 11(D_3)-methyloctadec-12-enoic acid was thus obtained after growing of the bacterial strain *Oceanicaulis alexandrii* sp. AG4 in a medium supplemented with [methyl-D_3]L-methionine (Aldrich) [14, 18].

Hydrogenation

11(D_3)-methyloctadec-12-enoic acid was hydrogenated (under an atmosphere of H_2) in methanol with Pd/$CaCO_3$ (5% Pd, 10–20 mg/mg of extract) (Aldrich) as a catalyst for 12 h with magnetic stirring. After hydrogenation, the catalyst was removed by filtration and the filtrate was concentrated by rotary evaporation.

Methylation

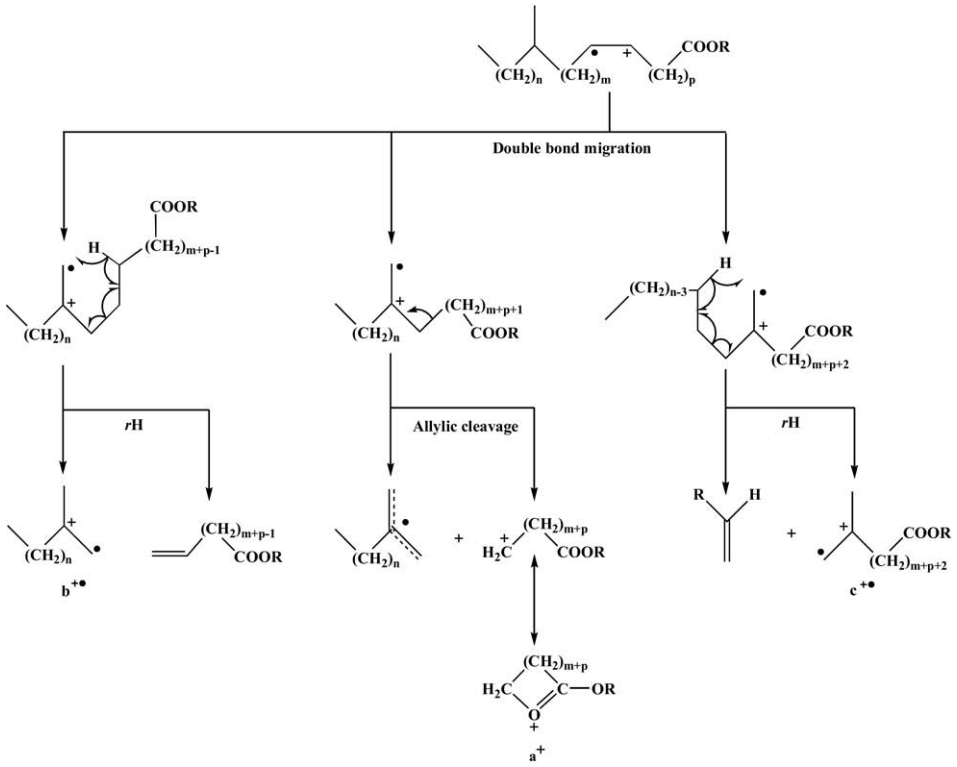
Lipid extracts were taken up in 2 mL of anhydrous methanolic hydrochloric acid (3N, St. Quentin Fallavier, France, Supelco) and heated at 80 °C for 1 h. After cooling, an excess of water was added and methyl esters were extracted three times with hexane-chloroform (4:1, vol/vol), dried over anhydrous Na_2SO_4, filtered, and concentrated using rotary evaporation.

Formation of Pyrrolidide Derivatives

Methyl esters were dissolved in 1 mL pyrrolidine. Then, 0.1 mL of acetic acid was added and the mixture was heated at 100 °C for 1 h. The amides so formed were taken up in dichloromethane and washed with diluted hydrochloric acid (to remove the excess of pyrrolidine) and with water. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated to obtain the required pyrrolidide derivatives.

Osmium Tetroxide Oxidation

Lipid extracts and OsO_4 (1:2, wt:wt) were added to a pyridine-dioxane mixture (1:8, vol/vol; 5 mL) and incubated for 1 h at room temperature. Then, 6 mL of Na_2SO_3 suspension (8.5 mL of 16% Na_2SO_3 in water-methanol, 8.5:2.5, vol/vol) was added and the mixture was again incubated for 1.5 h. The resulting mixture was gently acidified (pH 3) with HCl and extracted three times with dichloromethane (5 mL). The combined dichloromethane extracts were subsequently dried over anhydrous Na_2SO_4, filtered, and concentrated.



Scheme 1. Proposed formation pathways of ions a^+, $b^{+\bullet}$ and $c^{+\bullet}$ involving ionized double-bond migration to methylidene position and subsequent allylic cleavage and γ-hydrogen rearrangement.

Silylation

Compounds (1 mg) to be silylated were taken up in 300 μL of a mixture of pyridine and BSTFA (*N,O*-Bis(trimethylsilyl)trifluoroacetamide; Supelco) (2:1, vol/vol) and allowed to react at 50 °C for 1 h. After evaporation to dryness (to eliminate pyridine), the residue was dissolved in ethyl acetate (2 mL/mg) and BSTFA (0.1 mL) (to avoid desilylation) and analyzed by gas chromatography/mass spectrometry (GC/MS).

Mass Spectrometry

Analyses by gas chromatography/electron impact mass spectrometry were performed with a Hewlett Packard HP 5890 series II plus gas chromatograph connected to a HP 5972 mass spectrometer. The following operative conditions were employed: 30 m × 0.25 mm (i.d.) capillary column coated with SOLGEL-1 (SGE; film thickness, 0.25 μm); oven temperature programmed from 60 °C to 130 °C at 30 °C min^{-1} and then from 130 °C to 300 °C at 4 °C min^{-1}; carrier gas (He) pressure was maintained at 1.04 bar until the end of the temperature program and then programmed from 1.04 bar to 1.5 bar at 0.04 bar min^{-1}; injector (splitless) temperature, 250 °C; electron energy, 70 eV; source temperature, 170 °C; mass range, 50–700 Th; cycle time, 1.5 s.

Results and Discussion

The double-bond and branching positions of C_{15}-C_{18} *iso*- and anteiso-methyl-branched monounsaturated, 11-methyloctadec-12-enoic and 12-methyloctadec-11-enoic acid methyl and trimethylsilyl derivatives whose EI mass spectral fragmentations are examined in the present work were formally determined from EI mass spectra of their pyrrolidide derivatives. Confirmation of the position of double bonds involved GC-EIMS analyses of bis-trimethylsilyloxy derivatives obtained after OsO_4 oxidation and subsequent silylation. We also extended our conclusions to some EI mass spectra previously described in the literature [13, 16, 19].

EI mass spectrum of 11-methyloctadec-12-enoic acid methyl ester (Figure 1a) exhibits significant fragment ions at *m/z* 139, 140, 171, 179, 194, 211, and 226, whose formation by allylic cleavage and γ-hydrogen rearrangement may be well explained after migration of the Δ [12] double-bond to methylidene position (Scheme **1**). Indeed, in this case, allylic cleavage and γ-hydrogen rearrangement of the alkylester chain would mainly afford fragments ions a^+ at *m/z* 171 and $b^{+\bullet}$ at *m/z* 140, respectively; further loss of a neutral methanol molecule by the ion a^+ yielding the fragment ion at *m/z* 139. As previously proposed by Boon et al. [23], the driving force for the formation of ion a^+ could be its stabilization by cyclization with the ester group (Scheme **1**). This hypothesis was well supported by the lack of this ion in EI mass spectra of corresponding branched alkenes [23].

Table 1. Characteristic mass spectral fragments of some methyl-branched monounsaturated fatty acid methyl esters

Fatty acid methyl esters	a^+ *m/z* (%)[a]	$a^+ - CH_3OH$ *m/z* (%)	$a^+ - CH_3OH - H_2O$ *m/z* (%)	$b^{+\bullet}$ *m/z* (%)	$c^{+\bullet}$ *m/z* (%)	$c^{+\bullet} - CH_3OH$ *m/z* (%)	$c^{+\bullet} - CH_3OH - CH_3$ *m/z* (%)	$c^{+\bullet} - CH_3$ *m/z* (%)
16-Methylheptadec-9-enoic acid	241 (2)	209 (5)	191 (3)					
15-Methylhexadec-9-enoic acid	227 (18)	195 (20)	177 (14)					
14-Methylpentadec-9-enoic acid	213 (12)	181 (10)	163 (9)					
13-Methyltetradec-9-enoic acid	199 (10)	167 (14)	149 (12)					
14-Methylhexadec-9-enoic acid	213 (11)	181 (8)	163 (6)					
11-Methyloctadec-12-enoic acid	171 (9)	139 (14)		140 (12)	226 (3)	194 (8)	179 (7)	211 (6)
7-Methylhexadec-6-enoic acid [22]	115 (21)	83 (51)			170 (3)	138 (50)	123 (14)	155 (6)
7-Methylhexadec-8-enoic acid [16]	115 (32)	83 (45)			170 (2)	138 (62)	123 (22)	155 (5)
7-Methylhexadec-7-enoic acid [15]	115 (36)	83 (33)			170 (5)	138 (96)	123 (18)	155 (13)
6-Methylnonadec-5-enoic acid	101 (10)	69 (35)	51 (3)		156 (22)	124 (49)	109 (18)	141 (4)
4-Methylhexadec-11-enoic acid [13]	73 (22)				128 (78)	96 (43)	81 (23)	113 (5)
4-Methyloct-3-enoic acid [20]	73 (7)				128 (35)	96 (100)	81 (54)	113 (7)
4-Methyloct-2-enoic acid [20]	73 (6)				128 (83)	96 (100)	81 (46)	113 (20)
10-Methyleneoctadecanoic acid [26]	157 (36)	125 (43)	107 (7)	154 (5)	212 (2)	180 (25)	165 (24)	197 (10)

[a]Relative percentage.

The highest intensities of ions a^+ and ($a^+ - CH_3OH$) observed in the case of 7-methyl branched monounsaturated fatty acid methyl esters (where cyclization of the ion a^+ results in the formation of a well stabilized six-membered ring) (Table 1) are also in good agreement with this hypothesis. On the other side of the

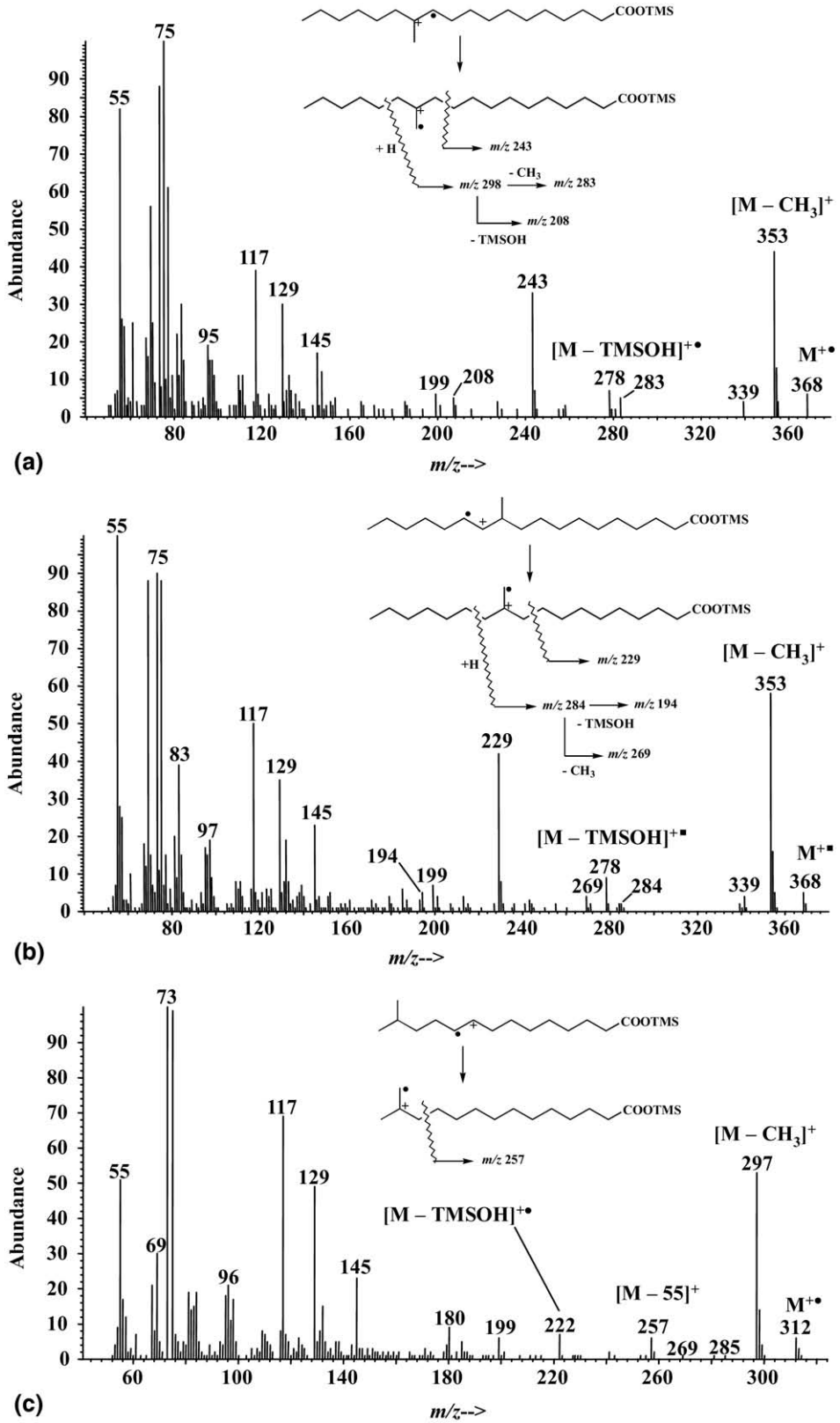


Figure 2. EI mass spectra of (**a**) 12-methyloctadec-11-enoic, (**b**) 11-methyloctadec-12-enoic, and (**c**) 13-methyltetradec-9-enoic acid trimethylsilyl esters.

molecule, γ-hydrogen rearrangement of the alkyl chain would result in the formation of a fragment ion $c^{+\bullet}$ at *m/z* 226, which could undergo subsequent losses of methanol molecule and/or methyl radical affording the ions at *m/z* 211 ($c^{+\bullet} - CH_3$), 194 ($c^{+\bullet} - CH_3OH$), and 179 ($c^{+\bullet} - CH_3 - CH_3OH$) (Figure 1a). The involvement of such double-

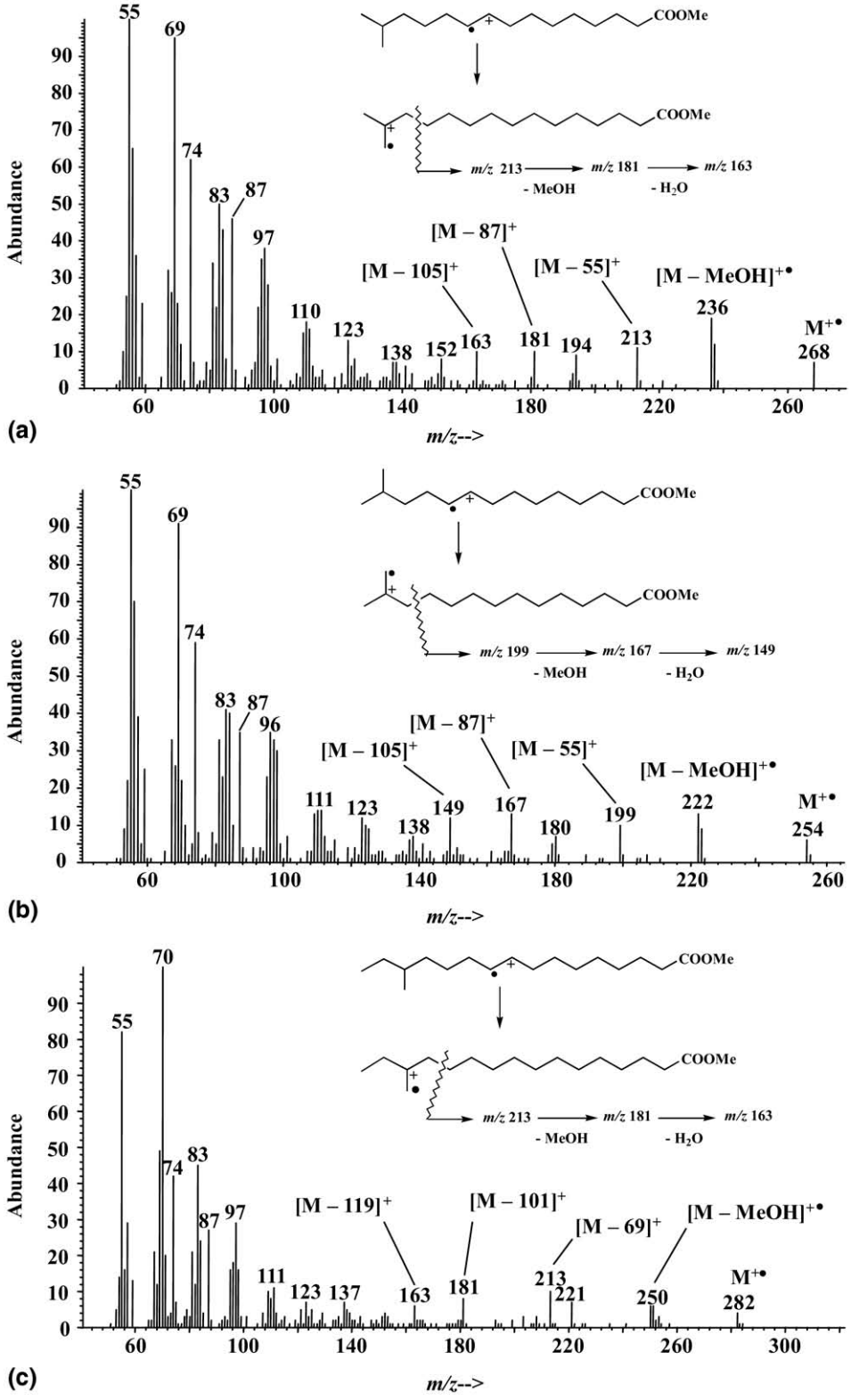


Figure 3. EI mass spectra of (**a**) 14-methylpentadec-9-enoic, (**b**) 13-methyltetradec-9-enoic, and (**c**) 14-methylhexadec-9-enoic acid methyl esters.

bond migration is well supported by the presence of a strong fragment ion at *m/z* 229 corresponding to ion a^+ in the EI mass spectrum of 11-methyloctadec-12-enoic acid trimethylsilyl ester (Figure 2b).

It was previously established that substitution of alkene hydrogen by electron-donating alkyl groups stabilizes the charge. Indeed, proton affinity (PA) of CH_3-CH=CH_2 and CH_3-CH=CH-CH_3 is 7.8 eV, while PA of $(CH_3)_2C=CH_2$ and $(CH_3)_2C=CHCH_3$ is 8.5 eV [24]. A preferential migration of the ionized double-bond of branched monounsaturated fatty acid derivatives towards the branching seems thus logical. Branching at the double-bond is also well known to be particularly effective for inducing specific dissociation by the γ-hydrogen rearrangement with little preceding hydrogen scrambling [24, 25].

To confirm the involvement of this preferential double-bond migration, we produced 11(D_3)-methyloctadec-12-enoic acid from a culture of the bacterial strain *Oceanicaulis alexandrii* sp. AG4, supplemented with [methyl-D_3]L-methionine. The efficiency of the labeling was checked after hydrogenation of this acid and subsequent methylation. The EI mass spectrum of the 11(D_3)-methyloctadecanoic acid methyl ester thus obtained (Figure 1b) confirmed the success of the methyl group labeling. Further examination of the mass spectrum of 11(D_3)-methyloctadec-12-enoic acid methyl ester (Figure 1c) showed that the labeling resulted to the shift of the fragment ions $b^{+\bullet}$, $c^{+\bullet}$, ($c^{+\bullet} - CH_3$), ($c^{+\bullet} - CH_3OH$), and ($c^{+\bullet} - CH_3 - CH_3OH$) to *m/z* 143, 229, 214, 197, and 182, respectively; while the ion a^+ remained unchanged. These results well support the presence of a methylidene double-bond at the position 11 in the ionized molecule.

An additional support of this preferential double-bond migration to the methylidene position was obtained from the EI mass spectrum of 10-methyleneoctadecanoic acid methyl ester, which was previously described in the literature [26]. Indeed, the fragment ions a^+, ($a^+ - CH_3OH$), ($a^+ - CH_3OH - H_2O$), $b^{+\bullet}$, $c^{+\bullet}$, ($c^{+\bullet} - CH_3$), ($c^{+\bullet} - CH_3OH$) and ($\mathbf{c}^{+\bullet} - CH_3 - CH_3OH$) (Table 1) correspond to most of the major peaks exhibited by this mass spectrum attesting that in this case the fragmentation of the ionized initial methylidene group takes place without significant double-bond migration.

Similar double-bond migration to methylidene positions allowed us to explain the formation of significant fragment ions in EI mass spectra of several other methyl-branched monounsaturated fatty acid methyl (Figure 3, Table 1) and trimethylsilyl (Figure 2, Table 2) derivatives. Such preferential migration appeared to intervene significantly in the case of fatty acid derivatives where the methyl branch is located between the penultimate and the C_4 positions (relative to the ester group). The particularly high intensity of the peak at *m/z* 128 corresponding to the ion $c^{+\bullet}$ in EI mass spectra of 4-methyl-branched monounsaturated fatty acid derivatives (Table 1) results probably from the stabilization of this ion by six-membered cyclization (Scheme **2**). In contrast, fragment ions resulting from cleavages of ionized methylidene group (which compete in this case with the strongly favored β- and γ-cleavages of the ester group) appeared to be very weak in EI mass spectra of 3- and 2-methyl-branched monounsaturated fatty acid derivatives [17, 20, 21, 27, 28].

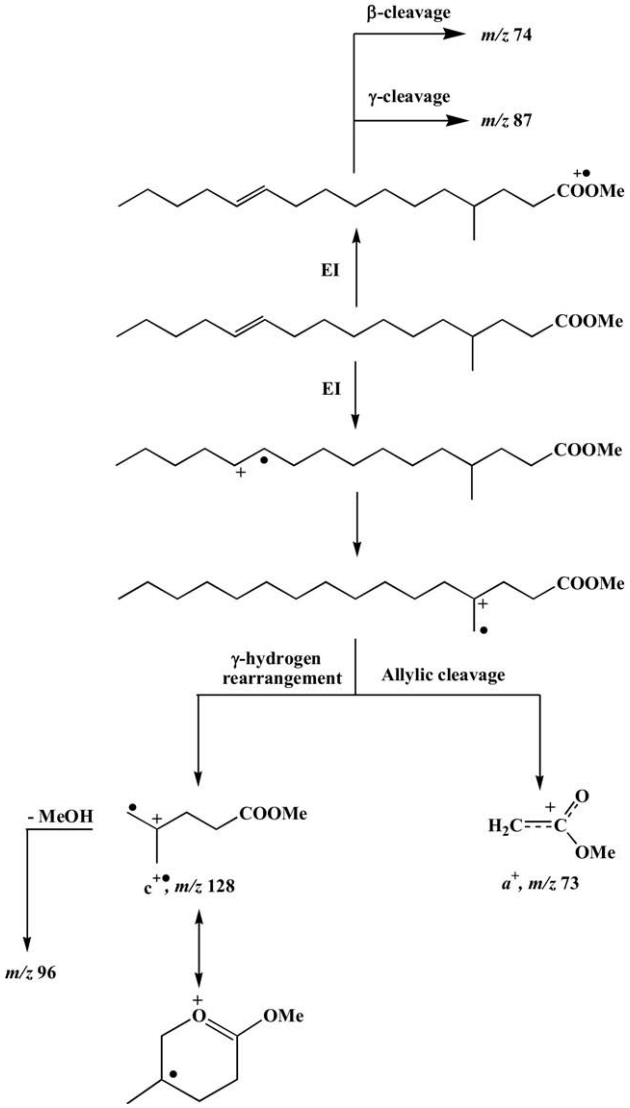
This preferential formation of ionized methylidene group seems to act whatever the double-bond position. However, it may be noted that double-bond migration is relatively limited during EI mass fragmentation of methyl-branched Δ [5] unsaturated fatty acid derivatives (Table 2). Indeed, EI mass spectra of these compounds are strongly dominated by fragment ions resulting from McLafferty rearrangement due to allylic activation by the Δ [5] double-bond of the itinerant γ-hydrogen.

Fragment ions at M − 55 (a^+), M − 87 ($a^+ - CH_3OH$) and M − 105 ($a^+ - CH_3OH - H_2O$) were previously proposed as characteristic for monounsaturated iso-methyl-branched fatty acid methyl esters and fragments at M − 69 (a^+), M − 101 ($a^+ - CH_3OH$) and M − 119 ($a^+ - CH_3OH - H_2O$) for monoenoic anteiso-methyl-branched fatty acid methyl esters [19, 23]. The results obtained in the course of the present work well support the proposal of these authors. However the use of fragment ions arising from EI fragmentation of the ionized methylidene group (formed after preferential double-bond migration) to characterize branched monounsaturated fatty acids is not limited to monounsaturated iso- and anteiso-branched fatty acid methyl esters. It could be extended to all the monounsaturated

Table 2. Characteristic mass spectral fragments of some methyl-branched monounsaturated fatty acid trimethylsilyl esters

Fatty acid trimethylsilyl esters	a^+ *m/z* (%)[a]	$c^{+\bullet}$ *m/z* (%)	$c^{+\bullet} - CH_3OH$ *m/z* (%)	$c^{+\bullet} - CH_3$ *m/z* (%)
16-Methylheptadec-9-enoic acid	299 (6)			
16-Methylheptadec-11-enoic acid	299 (5)			
14-Methylpentadec-9-enoic acid	271 (6)			
13-Methyltetradec-9-enoic acid	257 (6)			
12-Methyltetradec-9-enoic acid	243 (10)			
11-Methyloctadec-12-enoic acid	229 (43)	284 (3)	194 (6)	269 (5)
12-Methyloctadec-11-enoic acid	243 (34)		208 (6)	283 (6)
6-Methylnonadec-5-enoic acid	159 (7)	214 (4)	124 (15)	199 (2)

[a]Relative percentage.



Scheme 2. Proposed fragmentation pathways of 4-methylhexadec-11-enoic acid methyl ester.

fatty acid methyl and trimethylsilyl derivatives possessing a methyl branch localized between the penultimate and the C_4 positions. Owing to the involvement of this double-bond migration process, differentiation among monounsaturated fatty acid isomers that possess the methyl-branch at the same location but have double-bond at different places is not possible without additional treatments.

Conclusions

While the double-bond of monounsaturated linear fatty acid methyl and trimethylsilyl esters migrate to any position when the alkyl chain is ionized in the mass spectrometer, this is not the case during the ionization of monounsaturated methyl-branched fatty acid methyl and trimethylsilyl esters. Indeed, in this case, migration of the ionized double-bond appeared to be strongly favored to methylidene position, which undergoes subsequent allylic cleavage and γ-hydrogen rearrangement. The involvement of such a preferential migration was confirmed by deuterium labeling and examination of EI mass spectra of several monounsaturated methyl-branched fatty acids derivatives. The fragment ions resulting from the fragmentation of the ionized methylidene group thus formed will be very useful to characterize branching positions of monounsaturated fatty acid methyl and trimethylsilyl derivatives possessing a methyl branch localized between the penultimate and the C_4 positions, without additional treatment. Due to the involvement of the proposed specific double-bond migration, great care should be taken during the interpretation of EI mass spectra of methyl-branched monounsaturated fatty acid methyl and trimethylsilyl derivatives.

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