Elucidation of the Double-Bond Position of Long-Chain Unsaturated Fatty Acids by Multiple-Stage Linear Ion-Trap Mass Spectrometry with Electrospray Ionization

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Linear ion-trap (LIT) MS^2 mass spectrometric approach toward locating the position of double bond(s) of unsaturated long-chain fatty acids and toward discerning among isomeric unsaturated fatty acids as dilithiated adduct ($[M - H + 2Li]^+$) ions are described in this report. Upon resonance excitation in a LIT instrument, charge-remote fragmentation that involves β-cleavage with γ-H shift (McLafferty rearrangement) is the predominant fragmentation pathway seen for the $[M - H + 2Li]^+$ ions of monoenoic long-chain fatty acids. The fragmentation process results in a dilithiated product ion of terminally unsaturated fatty acid, which undergoes consecutive McLafferty rearrangement to eliminate a propylene residue, and gives rise to another dilithiated adduct ion of terminally unsaturated fatty acid. In addition to the above-cited fragmentation process, the $[M - H + 2Li]^+$ ions of homoconjugated dienoic long-chain fatty acids also undergo α-cleavage(s) with shift of the allylic hydrogen situated between the homoconjugated double bonds to the unsaturated site. These fragmentation pathways lead to two types of C—C bond cleavages that are allylic (α-cleavage) or vinylic, respectively, to the proximal C—C double bond, resulting in two distinct sets of ion series, in which each ion series is separated by a —CH_2CH=CH— (40 Da) residue. These latter fragmentations are the predominant processes seen for the polyunsaturated long-chain fatty acids. The spectrum feature dependent on the position of unsaturated double bond(s) affords unambiguous assignment of the position of double bond(s) of long-chain unsaturated fatty acids. (J Am Soc Mass Spectrom 2008, 19, 1673–1680) © 2008 American Society for Mass Spectrometry

Fast-atom bombardment (FAB) in combination with high-energy collisionally activated dissociation (CAD) tandem sector mass spectrometric analyses of the cationic adduct ions of unsaturated fatty acids with various metal ions, including lithium, calcium, and barium, yield product-ion mass spectra informative for identifying their double-bond location [1–4]. These spectra contain an ion series representing cleavage of consecutive C—C single bonds in the fatty acid chain, and then interrupted by a gap at the location of the double bond. Therefore, the gap is framed by two abundant ions on either side, leading to location of the double bond. Similar spectral features permit assignment of locations of other modifications of fatty acid structure, including branch points and hydroxyl or oxo moieties [5–8]. The mechanism underlying the fragmentation processes was coined as charge-remote fragmentation (CRF) and was thought to be mainly a high-energy fragmentation process [7]. An abundance of published literature discusses the fragmentation processes of long-chain fatty acids. For example, Harvey proposed a "charge-assisted process," a new vision of the mechanism apart from the charge-remote process, to account for ions of the $[M - C_nH_{2n+2}]$ series found in the positive-ion and negative-ion high-energy CAD spectra of fatty acids when ionized as closed-shell ($[M - H]^-$ or $[M + X]^+$) species [9 and references therein].

However, the classic fragmentation processes were also evidenced under low-energy CAD. For example, Hsu and Turk reported the CRF for cleavage of the fatty acyl substituent in sulfatides, leading to complete structural characterization including location of the position of double bond of the fatty acyl moiety using tandem quadrupole and quadrupole ion-trap instruments with electrospray ionization (ESI) in negative-ion mode [10].

By contrast, the mechanisms underlying the fragmentation processes under low-energy CAD in a tandem quadrupole instrument seen for the dilithiated adduct ions of unsaturated fatty acids generated by ESI involve mainly rearrangement of the highly labile α-allylic hydrogen to the site of the unsaturated bond. The structural information from the product-ion spectra

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1044-0305/08/$32.00
doi:10.1016/j.jasms.2008.07.007

Published online July 16, 2008
Received March 31, 2008
Revised July 1, 2008
Accepted July 8, 2008

affords assignment of the position of double bond(s) and distinguishes among isomeric unsaturated fatty acids [11]. In this study, we used multiple-stage linear ion-trap (LIT) mass spectrometry to revisit the fragmentation processes of the dilithiated adduct ions of unsaturated fatty acids generated by ESI. In addition to the major fragmentation processes involving rearrangement of the α-allylic hydrogen, as previously obtained with a tandem quadrupole instrument, ions arising from β-cleavage with rearrangement of γ-H (McLafferty rearrangement) [12] were also observed. Our results indicated that the major fragmentation pathways all involved rearrangement processes, leading to a unique product-ion spectrum that affords unambiguous assignment of the position of double bond(s). With subsequent use of multiple-stage mass spectrometry as described in the companion article [13], the insight into the mechanism of the fragmentation processes from this study paves the way to a complete structural characterization of glycerophospholipids, including assignment of the double-bond position of the fatty acid residues of the molecules.

Experimental

Materials

All fatty acids including 8,11,14-eicosatrienoic acid ($\Delta^{8,11,14}$20:3), 11,14,17-eicosatrienoic ($\Delta^{11,14,17}$20:3), 11-eicosenoic acid (Δ^{11}20:1), 11,14-eicosadienoic acid ($\Delta^{11,14}$20:2), 9-octadecenoic acid (Δ^{9}18:1), 6-octadecenoic acid (Δ^{6}18:1), 11-octadecenoic acid (Δ^{11}18:1), 9,12-octadecadienoic (linoleic acid, $\Delta^{9,12}$18:2), 9,12,15-octadecatrienoic acid (α-linolenic acid, $\Delta^{9,12,15}$18:3), 6,9,12-octadecatrienoic acid (gamma linolenic acid, $\Delta^{6,9,12}$18:3), 5,8,11,14-eicosatetraenoic acid (arachidonic acid, $\Delta^{5,8,11,14}$20:4), 7,10,13,16-docosatetraenoic acid ($\Delta^{7,10,13,16}$22:4), and 4,7,10,13,16,19-docosahexaenoic acid ($\Delta^{4,7,10,13,16,19}$22:6) were purchased from Nuchek Prep (Elysian, MN, USA). All other reagents were of spectroscopic grade and were purchased from Fisher Chemical (St. Louis, MO, USA).

Methods

Low-energy CAD tandem mass spectrometry experiments were conducted on a Finnigan (San Jose, CA, USA) linear ion-trap (LIT) mass spectrometer (MS) with the Xcalibur operating system. Fatty acid standards were dissolved in chloroform/methanol (1/4, vol/vol) at a final concentration of 10 pmol/μL and lithium hydroxide was then added to achieve a final [Li^+] of 1 mM. Lipid solution was infused (2 μL/min) to the ESI source, where the skimmer was set at ground potential, the electrospray needle was set at 4.5 kV, and temperature of the heated capillary was 300 °C. The automatic gain control of the ion trap was set to 5×10^4, with a maximum injection time of 400 ms. Helium was used as the buffer and collision gas at a pressure of 1×10^{-3} mbar (0.75 mTorr). The MS^n experiments were carried out with an optimized relative collision energy ranging from 20 to 25% and with an activation q value at 0.25, and the activation time at 30–50 ms to leave a minimal residual abundance of precursor ion (around 20%). Mass spectra were accumulated in the profile mode, typically for 3–10 min for MS^n (n = 2, 3, and 4) spectra. The mass resolution of the instrument was tuned to 0.6 Da at half peak height.

Nomenclature

We use a convention established by others [3] when describing relative locations among positions in a fatty acid chain. The terms "proximal" and "distal" reflect the relative proximity of other positions to the carboxylate moiety. For example, if the first C=C double bond in a fatty acid chain occurs between C(5) and C(6), then C(4) is considered proximal to the double bond, i.e., on the side of the double bond closer to the carboxylate, and C(7) is distal to the double bond.

Results and Discussion

MS^2 Spectra of the $[M - H + 2Li]^+$ Ions of the Fatty Acid Standards with Various Unsaturated Double Bonds

LIT MS^2 spectra of the isomeric monoenoic fatty acids. Although tandem quadrupole mass spectra arising from the $[M - H + 2Li]^+$ ions of long-chain fatty acids are useful for differentiation among isomers that differed by the location of double bonds [11], the LIT MS^2 product-ion spectra of the same ion species provide structural information that is readily applicable for locating the position of double bonds. For example, the LIT MS^2 spectrum of the $[M - H + 2Li]^+$ ion of Δ^9-18:1-fatty acid at *m/z* 295 (Figure 1a) is dominated by the ion at *m/z* 197, arising from cleavage of the C(11)—C(12) bond, along with the ion at *m/z* 141, arising from cleavage of the C(7)—C(8) bond. Interestingly, the ions from the similar cleavages were seen at *m/z* 195 and 141 in a high-energy CAD tandem mass spectrum obtained with a tandem sector instrument [14]. The ion at *m/z* 197 may arise from a CRF process with participation of the γ-hydrogen at C(13), which undergoes a McLaffery rearrangement to a double bond with β-cleavage [12], leading to a dilithiated terminal alkenyl carboxylic acid cation (Scheme **1**, route *a*), whereas the similar fragmentation process with the participation of the γ-hydrogen at C(6) leads to formation of the ion at *m/z* 141 (Scheme **1**, route *b*). The ion at *m/z* 99, which was the most prominent ion seen in the tandem quadrupole product-ion spectrum [11], may arise from further elimination of a CH_2=CH—CH_3 (42 Da) residue via a similar fragmentation process, whereas the consecutive losses of a CH_2=CH—CH_3 (42 Da) residue from the ion at *m/z* 197 leads to formation of the ions at *m/z* 155 and 113, respectively (Scheme **1**).

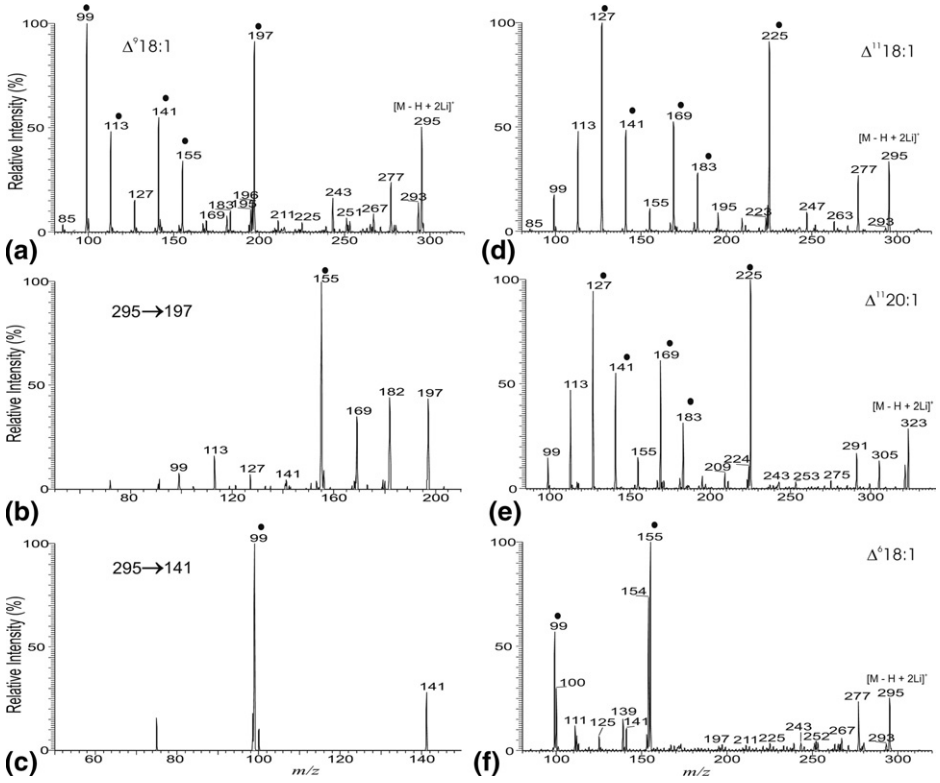
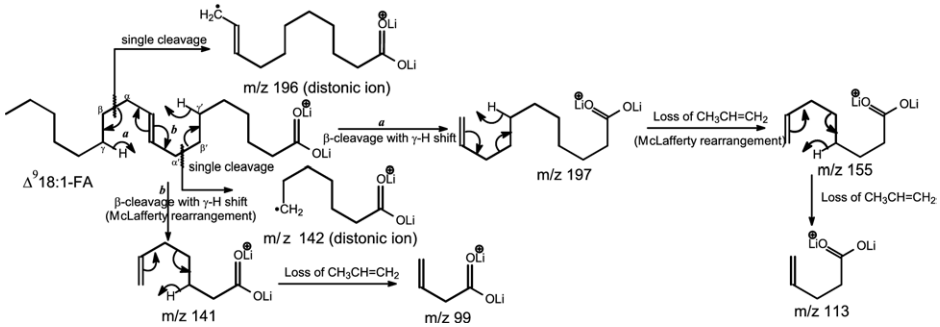


Figure 1. The LIT MS^2 spectrum of the $[M - H + 2Li]^+$ ion of $\Delta^9$18:1-fatty acid at *m/z* 295 (**a**), the MS^3 spectrum of the ion at *m/z* 197 (295 → 197) (**b**), and the MS^3 spectrum of the ion at *m/z* 141 (295 → 141) (**c**); the LIT MS^2 spectrum of the $[M - H + 2Li]^+$ ions of Δ^{11}18:1-fatty acid at *m/z* 295 (**d**), of Δ^{11}20:1-fatty acid at *m/z* 323 (**e**) and of Δ^{11}18:1-fatty acid at *m/z* 295 (**f**) are also shown. The ions labeled with "●" arise from the CRF processes with concurrence of β-cleavage and γ-H shift.

These fragmentation pathways leading to consecutive losses of a 42-Da residue were supported by the LIT MS^3 spectrum of the ion at *m/z* 197 (295 → 197, Figure 1b), 141 (295 → 141, Figure 1c), and 155 (not shown).

In addition to the aforementioned ions that derived from β-cleavage with γ-hydrogen shift, the ions probably arising from cleavage of the C—C bonds that are α-positioned to the double bond were seen at *m/z* 196 [cleavage of the C(11)—C(12) bond] and at *m/z* 142 [cleavage of the C(6)—C(7) bond]. These ions are distonic ions and the charge-assisted processes that have been previously proposed may account for their formation [10]. The observation of the 197/141 ion pair plus the presence of the unique distonic ion pairs of *m/z* 196/142 readily provide information that locates the double bond at C(9).

Similar fragmentation processes were seen for Δ^{11}18:1-fatty acid isomer, of which the LIT MS^2 spectrum of the $[M - H + 2Li]^+$ ion of *m/z* 295 (Figure 1d) is featured by the ion pairs at *m/z* 225/169, arising from the fragmentation processes that involve β-cleavage with γ-hydrogen, along with the distonic ion pair at *m/z* 224/168 arising from cleavage of the C(13)—C(14) and C(9)—C(10) bonds, respectively. These ions together with the ions at *m/z* 183 (225 − 42) and 141 (183 − 42) from consecutive losses of a CH_2=CH—CH_3 residue

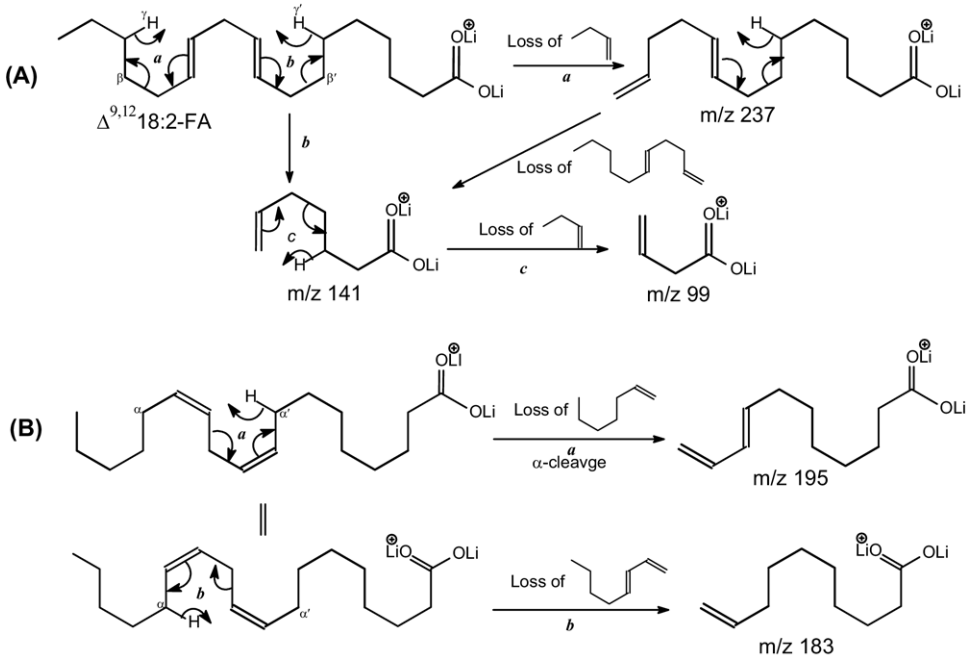


Scheme 1. The fragmentation mechanisms proposed for the $[M - H + 2Li]^+$ ion of $\Delta^9$18:1-FA.

from *m/z* 225, and the ion at *m/z* 127 (169 − 42) from *m/z* 169 by a similar loss indicate that the double bond is situated at C(11). The above-cited fragmentation processes leading to assignment of the position of the double bond are further supported by the LIT MS^2 spectrum of the Δ^{11}20:1-fatty acid at *m/z* 323 (Figure 1e), in which the fragment ions leading to structural assignment are nearly identical, consistent with the fact that the double bond is located at C(11). Similarly, the prominent ion pair at *m/z* 155/99 and the distonic ion pair at *m/z* 154/100 were seen in the LIT MS^2 spectrum of the $[M - H + 2Li]^+$ ions of isomeric $\Delta^6$18:1-fatty acid at *m/z* 295 (Figure 1f), leading to assignment of the structure of $\Delta^6$18:1.

LIT MS^2 spectra of dienoic fatty acids. The ions at *m/z* 237 and 141 arising from the mechanisms that involve the rearrangement of the γ-hydrogen to the outer side of the double bond (Scheme **2A**), similar to that described in Scheme **1**, were also seen in the LIT MS^2 spectra of the $[M - H + 2Li]^+$ ions of $\Delta^{9,12}$18:2-fatty acid at *m/z* 293 (Figure 2a). These ions were seen at *m/z* 237 (Scheme **2A**, route *a*), *m/z* 141 (route *b*), and *m/z* 99 (route *c*). However, the distonic ions expected at *m/z* 236 and 142 were not observed. The spectrum (Figure 2a) is dominated by the ion at *m/z* 195, which may arise from cleavage of the C(11)—C(12) bond (α-cleavage) with rearrangement of the allylic hydrogen at C(8) to eliminate a 1-heptene residue, giving rise to a dilithiated ion of a terminal conjugated fatty acid residue (Scheme **2B**, route *a*). This ion reflects cleavage of the C(11)—C(12) bond that is allylic to the first C=C double bond lying on the side of the double bond farther away from the carboxylate (i.e., distal to the double bond) and provides additional information for locating the position of double bonds. Similar fragmentation process involving the distal allylic hydrogen at C(14) that is in the α-position (allylic) to the outer double bond leads to the formation of the ion at *m/z* 183 (Scheme **2B**, route *b*). The above-cited fragmentation pathways were further supported by the LIT MS^2 spectrum of the $[M - H + 2Li]^+$ ions of $\Delta^{11,14}$20:2-fatty acid at *m/z* 321 (Figure 2b). The spectrum contains the ions at *m/z* 265, 169, 223, and 211, which are 28 Da ($[CH_2]_2$) heavier than the analogous ions at *m/z* 237, 141, 195, and 183 seen for $\Delta^{9,12}$18:2-fatty acid (Figure 2a), reflecting the structure of $\Delta^{11,14}$20:2. The ions arising from the fragmentation processes, as depicted in Scheme **2B**, were also seen in the tandem quadrupole mass spectra [11] and the analogous ions arising from the fragmentation processes were seen in all of the LIT MS^2 spectra of polyunsaturated fatty acids as shown in the following text.

LIT MS^2 spectra of trienoic fatty acids. As seen in Figure 3a, the LIT MS^2 spectrum of dilithiated $\Delta^{6,9,12}$18:3-fatty acid at *m/z* 291 is dominated by the ion at *m/z* 193, which arises from cleavage of the C(10)—C(11) bond with rearrangement of the highly labile allylic hydrogen at C(8), resulting in a stable conjugated ion of dilithiated $\Delta^{6,8,10}$11:3-fatty acid (Scheme **3A**, route *a*). Similar fragmentation process involving rearrangement of the allylic hydrogen at C(11) resulted in the formation of the ion at *m/z* 141 by cleavage of the C(7)—C(8) bond to eliminate a highly conjugated 1,3,5-nondecatriene (Scheme **3B**). Since the homoconjugated hydrogens at C(8) and C(11) (i.e., allylic to two double bonds) are the most labile, their shifts to the unsaturated sites [to C(12) and C(7)] may become more facile, resulting in the prominence of ions at *m/z* 193 and 141. By contrast, the ion at *m/z* 153 may arise from rearrangement of the less



Scheme 2. The fragmentation mechanisms proposed for the $[M - H + 2Li]^+$ ion of $\Delta^{9,12}$18:2-FA.

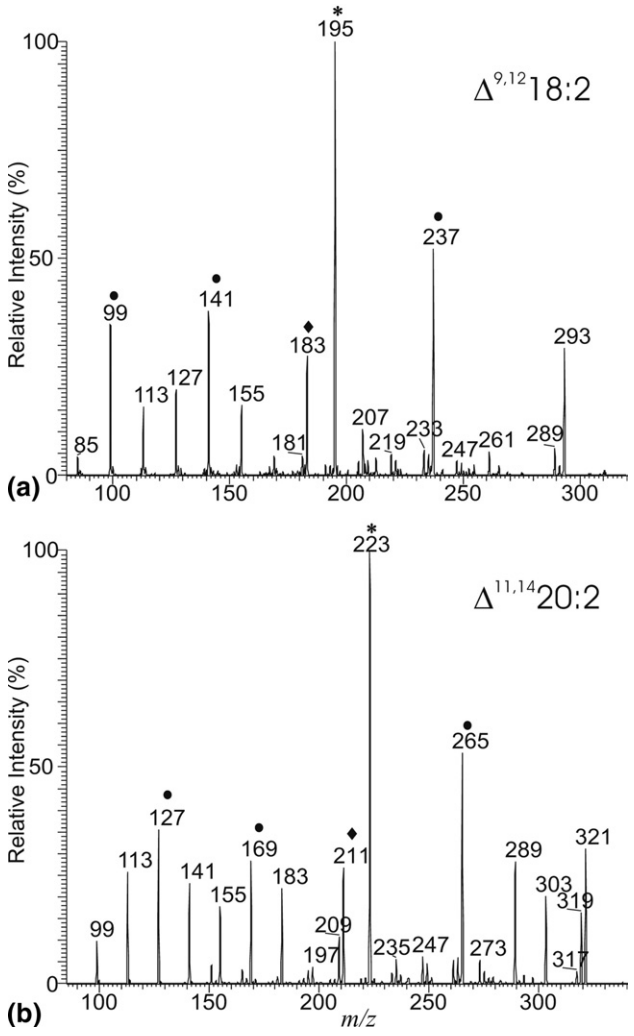


Figure 2. The LIT MS^2 spectrum of the $[M - H + 2Li]^+$ ions of $\Delta^{9,12}$18:2-fatty acid at *m/z* 293 (**a**), and of $\Delta^{11,14}$20:2-fatty acid at *m/z* 321 (**b**). The ions labeled with "♦" (vinyl cleavage) "*" (allylic cleavage) and "●" (*β*-cleavage) lead to locate the double bonds of the unsaturated long-chain fatty acids.

labile *α*-hydrogen at C(5), followed by cleavage of C(8)—C(9) bond to form a terminal conjugated ion of a dilithiated $\omega^{1,3}$8:2 (Scheme **3A**, route *b*), which is less conjugated (compared to the ion at *m/z* 193) and is of low abundance.

The above-cited fragmentation processes are further supported by the LIT MS^2 spectrum of the dilithiated $\Delta^{8,11,14}$20:3-fatty acid at *m/z* 319 (Figure 3c), which contains the prominent ion at *m/z* 221, arising from cleavage of the C(12)—C(13) bond to a highly conjugated dilithiated $\Delta^{8,10,12}$13:3-fatty acid ion, along with the ion at *m/z* 169 arising from loss of a 1,3,5-nondecetriene residue. The ion at *m/z* 181 arises from a similar cleavage of the C(10)—C(11) bond with arrangement of the *α*-hydrogen proximal to the first C=C bond. These ions are 28 Da heavier than the analogous ions at *m/z* 193, 141, and 153, respectively, seen for $\Delta^{6,9,12}$18:3. The results are consistent with the notion that both the $\Delta^{6,9,12}$18:3 and $\Delta^{8,11,14}$20:3 are ω^6 series fatty acids (i.e., $\omega^{6,9,12}$18:3- and $\omega^{6,9,12}$20:3-fatty acids, respectively).

The profiles of the IT MS^2 spectra of the $[M - H + 2Li]^+$ ions of $\Delta^{9,12,15}$18:3 at *m/z* 291 (Figure 3c) and of $\Delta^{11,14,17}$20:3-fatty acid at *m/z* 319 (Figure 3d) are also similar to those of $\Delta^{6,9,12}$18:3 (Figure 3a) and of $\Delta^{8,11,14}$-20:3 (Figure 3b). The former spectrum (Figure 3c) contains the ions at *m/z* 235, 183, 195, and 141, which are 42 Da [representing a $(CH_2)_3$ chain] heavier than the analogous ions at *m/z* 193, 141, 153, and 99 seen for $\Delta^{6,9,12}$18:3, consistent with the fact that the compound is a $\Delta^{9,12,15}$18:3-fatty acid, an $\omega^{3,6,9}$18:3-fatty acid of which the proximal double bond is $(CH_2)_3$ further distant to the carboxylate group, as compared to $\Delta^{6,9,12}$18:3. The latter spectrum (Figure 3d) contains the feature ions at *m/z* 263, 211, 223, and 169, which are 42 Da ($[CH_2]_3$) heavier than the analogous ions seen at 221, 169, 181, and 127 seen for $\Delta^{8,11,14}$20:3, consistent with the $\Delta^{11,14,17}$20:3-fatty acid structure, which is also an $\omega^{3,6,9}$20:3-fatty acid.

LIT MS^2 spectra of polyunsaturated fatty acids. Arachidonic acid ($\Delta^{5,8,11,14}$20:4; $\omega^{6,9,12,15}$20:4) is also an ω^6-series fatty acid, similar to $\Delta^{8,11,14}$-20:3-fatty acid ($\omega^{6,9,12}$20:3). As shown in Figure 4a, the IT MS^2 spectrum of the dilithiated $\Delta^{5,8,11,14}$20:4-fatty acid at *m/z* 317 contains the ions at *m/z* 261, 219, 179, and 167, which are 2 Da lighter than the analogous ions at *m/z* 263, 221, 181, and 169 seen for $\Delta^{8,11,14}$20:3, consistent with the presence of an additional double bond located at C(5). The hydrogen atoms at C(7), C(10), and C(13) are homoconjugated allylic hydrogens (situated between two double bonds) and are labile. Again, the prominent ion at *m/z* 219 arises from rearrangement of the hydrogen at C(10), followed by cleavage of the C(13)—C(14) bond, leading to formation of a stable dilithiated adduct ion of a highly conjugated $\Delta^{5,8,10,12}$13:4, whereas the prominence of the ion at *m/z* 179 is attributed to the similar fragmentation process that involves the shift of the hydrogen at C(7), followed by cleavage of C(10)—C(11) bond to form a dilithiated $\Delta^{5,7,9}$10:3. This latter ion (i.e., *m/z* 179) is analogous to the ion at *m/z* 181 seen for $\Delta^{8,11,14}$20:3 (Figure 3b), but is significantly more affluent, consistent with the notion that the ion at *m/z* 181 represents a dilithiated ion of $\Delta^{7,9}$10:2, which is less conjugated than the dilithiated $\Delta^{5,7,9}$10:3 ion of *m/z* 179 and the *α*-hydrogen at C(7) in $\Delta^{8,11,14}$20:3 is a less labile allylic hydrogen (next to one double bond). Similarly, cleavage of C(7)—C(8) bond with C(4)—hydrogen shift leads to formation of a dilithiated ion of $\Delta^{4,6}$7:2-fatty acid ion at *m/z* 139, which is less conjugated and less prominent than the ion at *m/z* 179, whereas cleavage of C(6)—C(7) bond with rearrangement of homoconjugated C(10)—hydrogen results in *m/z* 127 due to loss of a highly conjugated 1,3,5,8-tetradecatetraene residue. The observation of these latter two ions supports the presence of the double bond at C(5), consistent with the notion that the compound is a $\Delta^{5,8,11,14}$20:4-fatty acid.

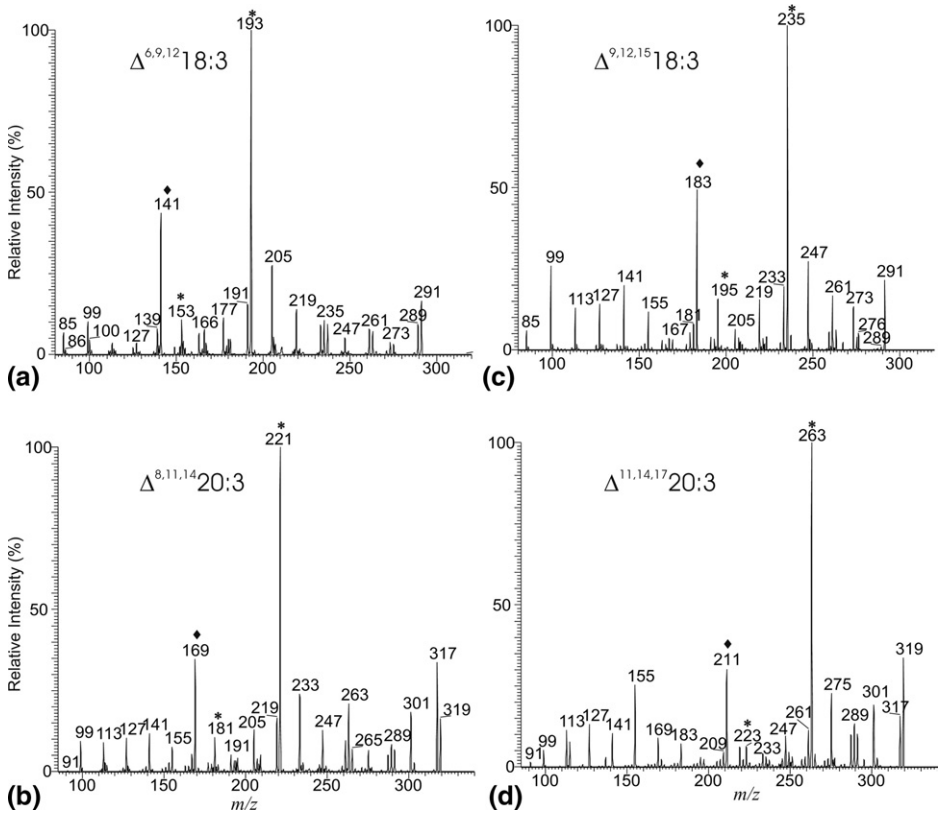
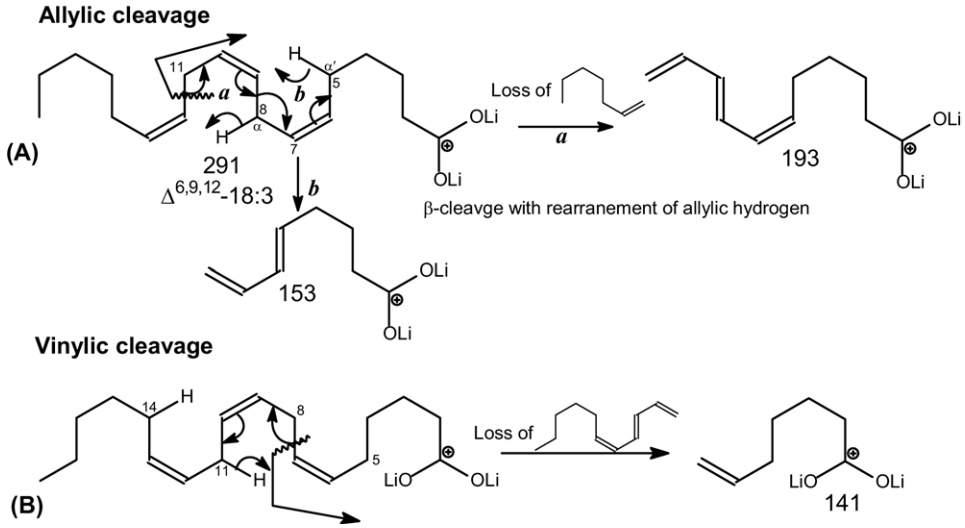


Figure 3. The LIT MS^2 spectrum of the $[M - H + 2Li]^+$ ions of $\Delta^{6,9,12}$18:3-fatty acid at *m/z* 291 (**a**), of $\Delta^{8,11,14}$18:3-fatty acid at *m/z* 291 (**b**), of $\Delta^{8,11,14}$20:3-fatty acid at *m/z* 319 (**c**), and of $\Delta^{8,11,14}$20:3-fatty acid at *m/z* 319 (**d**). Ions labeled with "♦" and "*" arise from vinyl and allylic cleavages, respectively.

The profile of the MS^2 spectrum of the $[M - H + 2Li]^+$ ions of $\Delta^{7,10,13,16}$22:4 at *m/z* 345 (Figure 4b) is nearly identical and ions informative for locating the double bonds are 28 Da ($[CH_2]_2$) higher than those seen for $\Delta^{5,8,11,14}$20:4 (Figure 4a). The results are consistent with the fragmentation processes and are in accord with the fact that $\Delta^{7,10,13,16}$22:4 is also an ω^6-series fatty acid, in which the closest double bond proximal to the carboxylate group is —CH_2CH_2— longer than that in $\Delta^{5,8,11,14}$20:4.

By contrast, the $\Delta^{4,7,10,13,16,19}$22:6 fatty acid (4, 7, 10, 13, 16, 19)-docosahexaenoic acid) contains five sets of homoconjugated hydrogen atoms at C(6), C(9), C(12), C(15), and C(18), along with less labile α-allylic hydro-



Scheme 3. The fragmentation mechanisms proposed for the $[M - H + 2Li]^+$ ion of $\Delta^{6,9,12}$18:3-FA.

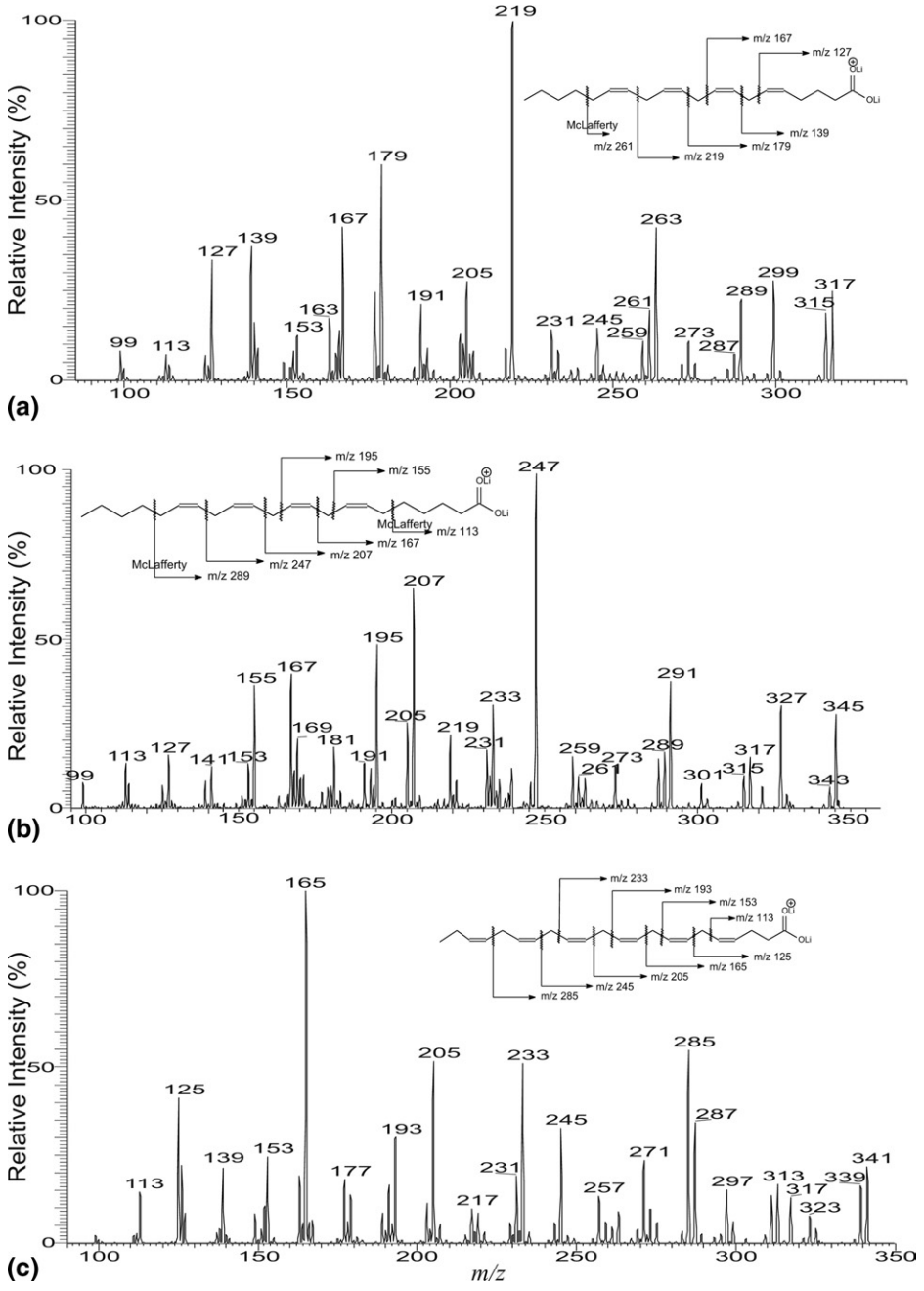


Figure 4. The LIT MS^2 spectrum of the $[M - H + 2Li]^+$ ions of $\Delta^{5,8,11,14}$20:4-fatty acid at *m/z* 317 (**a**), of $\Delta^{7,10,13,16}$22:4-fatty acid at *m/z* 345 (**b**), and of $\Delta^{4,7,10,13,16,19}$22:6-fatty acid at *m/z* 341 (**c**).

gen at C(3) and C(21). The fragmentation processes reflecting cleavages of the C—C bond distal to the α-carbons between double bonds (i.e., on the side of the bond farther away from the carboxylate) with rearrangement of the hydrogens at C(18), C(15), C(12), C(9), C(6), and C(3), respectively, lead to ions at *m/z* 325, 285, 245, 205, 165, and 125, respectively. The cleavages of the C—C bond proximal to the double bonds (vinyl cleavage) between double bonds (i.e., on the side of the bond closer the carboxylate) with rearrangement of the hydrogens at C(21), C(18), C(15), C(12), and C(9), respectively, resulting in the formation of the ions at *m/z* 273, 233, 193, 153, and 113, respectively. These latter ion series are less intense than those in the former series, consistent with the notion that the ions in the former series are highly conjugated dilithiated ions of terminally conjugated fatty acids as described earlier.

Conclusions

The $[M + Li]^+$ ion or the $[M - H]^-$ ion in the negative-ion mode is readily formed, when subjected to ESI. However, the LIT product-ion spectra from MS^n ($n = 2$, 3, or 4) on the $[M + Li]^+$ or the $[M - H]^-$ ion do

not provide structural information for locating the double bond(s) of long-chain fatty acids (data not shown). The charge fixation of the $[M + Li]^+$ ions is not strong enough that these ions dissociate in a way that the lithium cation is lost before the CRF processes with concurrence of shift of γ- or allylic hydrogen take place [3, 8], whereas the $[M - H - H_2O]^-$ and $[M - H - CO_2]^-$ ions arising from neutral loss of H_2O or CO_2 are the predominant ions seen in the MS^2 product-ion spectra of the $[M - H]^-$ ion of fatty acids, dependent on the degree of unsaturation. By contrast, the $[M + Li]^+$ adduct ions of unsaturated glycerophospholipids [13] and of unsaturated triacylglycerol (data not shown) undergo the similar CRF upon resonance excitation in a linear ion trap, yielding structural information for locating the double bond(s) of the long-chain fatty acid substituents. The use of multiple-stage LIT mass spectrometry for locating the unsaturated bond of unsaturated triacylglycerol is currently in progress.

Acknowledgments

This research was supported by US Public Health Service Grants P41-RR-00954, R37-DK-34388, P60-DK-20579, and P01-HL-57278, and P30-DK56341.

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