JOURNAL OF MASS SPECTROMETRY, VOL. 33, 461–472 (1998)

2-Alkylimidazoline Derivative to Control Fatty Acid Fragmentation upon Electron Impact and Electrospray Ionization

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A new derivative of the carboxyl group, *N*-methyl-2-alkylimidazoline, was introduced with the aim of improving the control of fatty acid fragmentation. Owing to the strongly basic character and the cyclic structure of this derivative, strong stabilization of the charge on the ionized group is obtained so that extensive radical and charge-remote fragmentation of the chain can be achieved with electron impact and electrospray ionization. © 1998 John Wiley & Sons, Ltd.

J. Mass Spectrom. **33**, 461–472 (1998)

KEYWORDS: fatty acids; derivatives; structure elucidation; electron impact; electrospray

INTRODUCTION

Branchings and unsaturation in fatty acids can be localized by two convenient mass spectrometric methods. One is based on derivatization of the carboxyl group and radical-induced fragmentation of the chain upon electron impact ionization. Derivatives used in this technique are amides, e.g. pyrrolidides,[1–3] or nitrogen heterocycles, e.g. β-picolinyl-esters,[4,5] 2-alkyltriazolopyridines[6] or 2-alkyl-4,4-dimethyloxazolines (DMOX derivatives).[7] The other method is based on charge-remote fragmentation[8–12] obtained by collisional activation of various ions derived from the carboxyl group, e.g. cationic adducts,[13] or derivatives of the acid, e.g. amides carrying a *p*-aminobenzenesulfonate[14] or a phosphonium group.[15] In addition, charge-remote fragmentation can be achieved from carboxylate anions, either produced directly by fast atom bombardment or electrospray,[16] or alternatively by electron attachment on pentafluorobenzyl esters and subsequent loss of the pentafluorobenzyl radical.[17] This technique is usually employed on magnetic sector instruments with high collisional energy.[8–10] However, for certain functional groups chain fragmentation has also been achieved in quadrupole instruments at low collision energies.[18–20]

All of the above techniques leave room for improvements where routine application is desired, be it in the preparation, in the chromatographic separation of mixtures, or in the chain fragmentation, as all three are important aspects of routine fatty acid analysis.

Of the various possibilities, the DMOX derivative appears to be most thoroughly studied and the easiest for routine applications.[21–27] As indicated above, this derivative has been used in connection with gas chromatography mass spectrometry (GC/MS) and electron impact (EI) ionization, where it shows, in addition to satisfactory behaviour in GC, a remarkably distinct fragmentation pattern, especially for polyunsaturated chains.

To our knowledge, the charge-remote fragmentation of this derivative upon any type of chemical ionization has not been investigated. Since the basicity of the oxazoline group is rather weak, it does not seem ideally suited for this type of analysis.

It appeared interesting to us to devise a derivative patterned after the oxazoline derivative, which is easily prepared, can be handled by gas and liquid chromatography (LC) and shows characteristic chain fragmentation upon *both* types of ionization techniques, i.e. as a radical cation upon EI and as a protonated species upon electrospray ionization (ESI).

There are two structural features necessary for such a derivative. One is a high proton affinity, which provides a strong driving force for hydrogen abstraction from the chain as the key step of chain fragmentation after electron impact.[28,29] The other is a stable protonated structure that is solidly linked to the chain with no easy cleavage within or at the functional group, so that no charge-induced fragmentation is competing with the desired charge-remote fragmentation of the chain.[19]

An amidine structure in the form of an imidazoline, being much more basic than an oxazoline, appeared to be a structure which could fulfil both requirements to a satisfactory extent. In addition, an imidazoline like an oxazoline can be easily prepared from acids and esters via the corresponding amides and can be handled by GC/MS and LC/MS.

Preliminary trials with ethane-1,2-diamine and *N*-methylethane-1,2-diamine (MEDA) as reagents led to the decision to develop the latter, resulting in the 2-alkyl-*N*-methylimidazoline (MIM) derivative. This derivative had the more favourable features with regard to preparation and GC.

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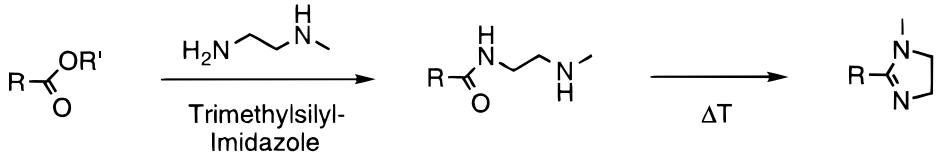
CCC 1076–5174/98/050461–12 $17.50

Received 11 December 1997
Accepted 9 February 1998



Scheme 1. Synthesis of the MIM derivative from fatty acids (R′ = H) or glycerides (R′ = glyceryl).

The preparation of the intermediate amide can be carried out by dissolving the glyceride or the fatty acid in neat MEDA, adding trimethylsilylimidazole as a catalyst[30] and heating at 80 °C for 3 h. Extracting the amide into toluene and heating this solution under slightly acidic conditions at 120 °C for 2 h produces the desired *N*-methyl-2-imidazoline in almost quantitative yield (Scheme 1).

With respect to synthesis, the proposed derivative has two advantages over the DMOX derivative. First, the reagent MEDA is a liquid at room temperature and a good solvent for lipids, and second, the cyclization requires much less drastic conditions. However, there is also a drawback in that slightly higher temperatures are required in the GC analysis.

Seven common fatty acids with saturated, monoolefinic or polyolefinic chains and one dibasic acid were selected for studying the fragmentation of their MIM derivatives upon EI and ESI (for structures, see Figs 1–3).

EXPERIMENTAL

A 1 mg amount of glyceride or fatty acid is dissolved in a mixture of 20 μl of *N*-methylethane-1,2-diamine (Fluka) (b.p. 115 °C) and 20 μl of trimethylsilylimidazole (Pierce).[30] After heating for 3 h at 80 °C the reaction mixture is diluted with 500 μl of water and left for a few minutes to hydrolyse the trimethylsilylamines. The mixture is extracted with 400 μl of toluene. (If undissolved material remains, the extraction should be performed with 400 μl of ethyl acetate and the solvent replaced by toluene after drying.) About 5 mg salicylic acid are added to the toluene solution and heated at 120 °C in a Reactivial (Pierce). Cyclization is complete after 2 h. The solution can be used directly for GC/MS or LC/MS analysis. If desired, the salicylic acid can be removed by shaking the toluene solution with aqueous sodium carbonate.

The derivatives of the C_{18}-α,ω-dicarboxylic acid were prepared as mixtures by partial reaction of the dimethyl ester with MEDA and 2-methyl-2-aminopropanol and subsequent cyclization. GC/MS was used for obtaining the EI mass spectra of the components of the resulting mixture and flow injection for the ESI mass spectra.

ESI mass spectra were obtained on an API III mass spectrometer (Perkin-Elmer SCIEX), using Ar at a density of (130–270) × 10^{13} atoms cm^{-2} and a collision energy between 30 and 60 eV. The samples were dissolved in a mixture of acetonitrile and 0.01 M aqueous ammonium acetate (1 : 1) at a concentration of about 10 μM, and introduced by flow injection into a stream of acetonitrile–water (1 : 1). The software used for data treatment was MULTIVIEW from Perkin-Elmer SCIEX.

EI mass spectra were obtained by GC/MS on a model 5989B instrument (Hewlett-Packard). The ionization energy was 70 eV and the temperature of the ion source was 250 °C. The software for data treatment was Masslib V8.4-C3 (Chemical Concepts, Weinheim, Germany).

RESULTS AND DISCUSSION

EI of MIM derivatives

Figure 1(a) and (b) show the EI mass spectra of the MIM derivatives of stearic acid and its ω-trideuteriated analogue. The fragmentation of the chain is similar to the well known fragmentation of fatty esters[29] and produces the complete series of chain fragments, starting at *m/z* 98, the product of the McLafferty reaction, and continuing with even-electron fragments up to M − methyl. Comparison of the two spectra shows that the fragmentation is almost exclusively due to loss of the terminal parts of the chain, and only a negligible portion is formed by excision of interior parts of the chain (the corresponding peaks at M − 29 Da and M − 43 Da in Fig. 1(b) are marked with arrows), in agreement with the observations on amides and *N*-heterocycles,[6] but in strong contrast to esters.[31–33] This is a favourable feature of the new derivative from the point of view of structure elucidation because it simplifies the localization of branchings, particularly for the distinction of iso- and anteiso-variants from straight chains.

In Fig. 2(a)–(f) a series of spectra of MIM derivatives of naturally occurring unsaturated fatty acids with different numbers of double bonds in various positions is presented. From the *m/z* values of the series of fragment peaks the position of each double bond can be determined. The spectra are remarkably similar to those of the DMOX derivatives,[7] showing peaks for all possible C—C bond cleavages in the chain, including those in vinylic positions. This similarity even extends to the peaks from fragments, where formal cleavage of a double bond is required. These peaks deserve special attention because they may give rise to ambiguities in the localization of the double bonds.

There are two types of such cleavages. The first type is represented, for example, by the peak at *m/z* 221 in Fig. 2(a), 193 in Fig. 2(b) and (d) or 203 and 283 in Fig. 2(f). Andersson and Holman[2] have previously identified such peaks in the spectra of acylpyrrolidines and also Huang and co-workers[22] in the spectra of DMOX derivatives.

These types of fragments are formed from double bonds more remote than position 6 from the functional group. The peaks occur at mass numbers which imply that the double bond has been shifted before fragmenta-

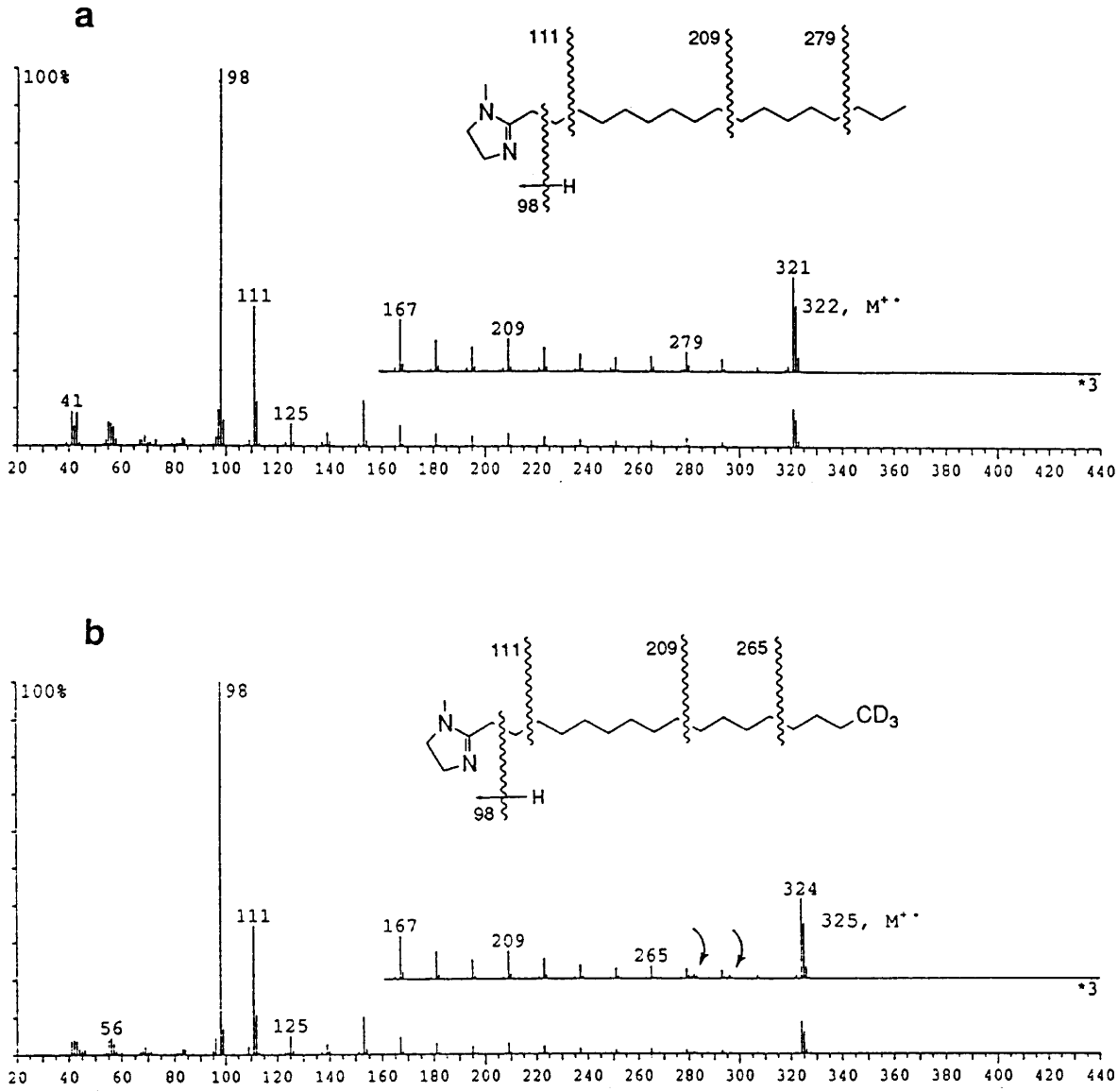


Figure 1. Electron impact mass spectra (70 eV) of the MIM derivative of (a) stearic acid and (b) ω-trideuteriostearic acid. The essential features of the fragmentation are indicated on the structures. The arrows mark fragments formed by extrusion of parts from the interior of the chain.

tion, so that a single bond is now at the original position of the double bond. This single bond is then cleaved as all other single bonds. If such a shift of the double bond is assumed, it must be concluded from the observed masses that it preferably occurs in the direction towards the functional group. This observation was cast as a rule by the authors mentioned above. The rule states that m/z of the observed peak always occurs at a separation of 12 Da from the next lower peak in the series, in contrast to the 14 Da seen in all other cases.

When the double bonds are closer to the functional group, e.g. in position 4, 5 or 6, then there appears another type of cleavage represented by intense peaks at even mass numbers corresponding to radical ions which formally require a straight cut through the double bond. For example, m/z 152 in Fig. 2(c) (double bond in position 6), m/z 138 in Fig. 2(e) (position 5) and m/z 124 in Fig. 2(f) (position 4) represent such ions. These peaks are highly characteristic of such double bonds, but the mechanism by which the fragments are formed is at present not well understood. Presumably in this case the double bond is also shifted towards the functional group, opening up the possibility of a McLafferty-type rearrangement.

A further observation must be mentioned which occurs only in the spectrum of the docosahexaenoic acid derivative [Fig. 2(f)]. There is an intense odd-electron fragment at m/z 218. Except for the radical ions originating in the McLafferty reaction and those from the formal cleavage of double bonds just mentioned, this is the only significant radical ion fragment in all the spectra examined. The corresponding ion has been observed also as a unique exception in the spectrum of the DMOX derivative of the same acid.[7] The reason for its occurrence remains obscure.

Figure 3(a)–(c) serve to show that the MIM derivative strongly controls the fragmentation even in the presence of other functional groups which normally dominate the fragmentation themselves e.g. the ester group or the DMOX group. In these figures three

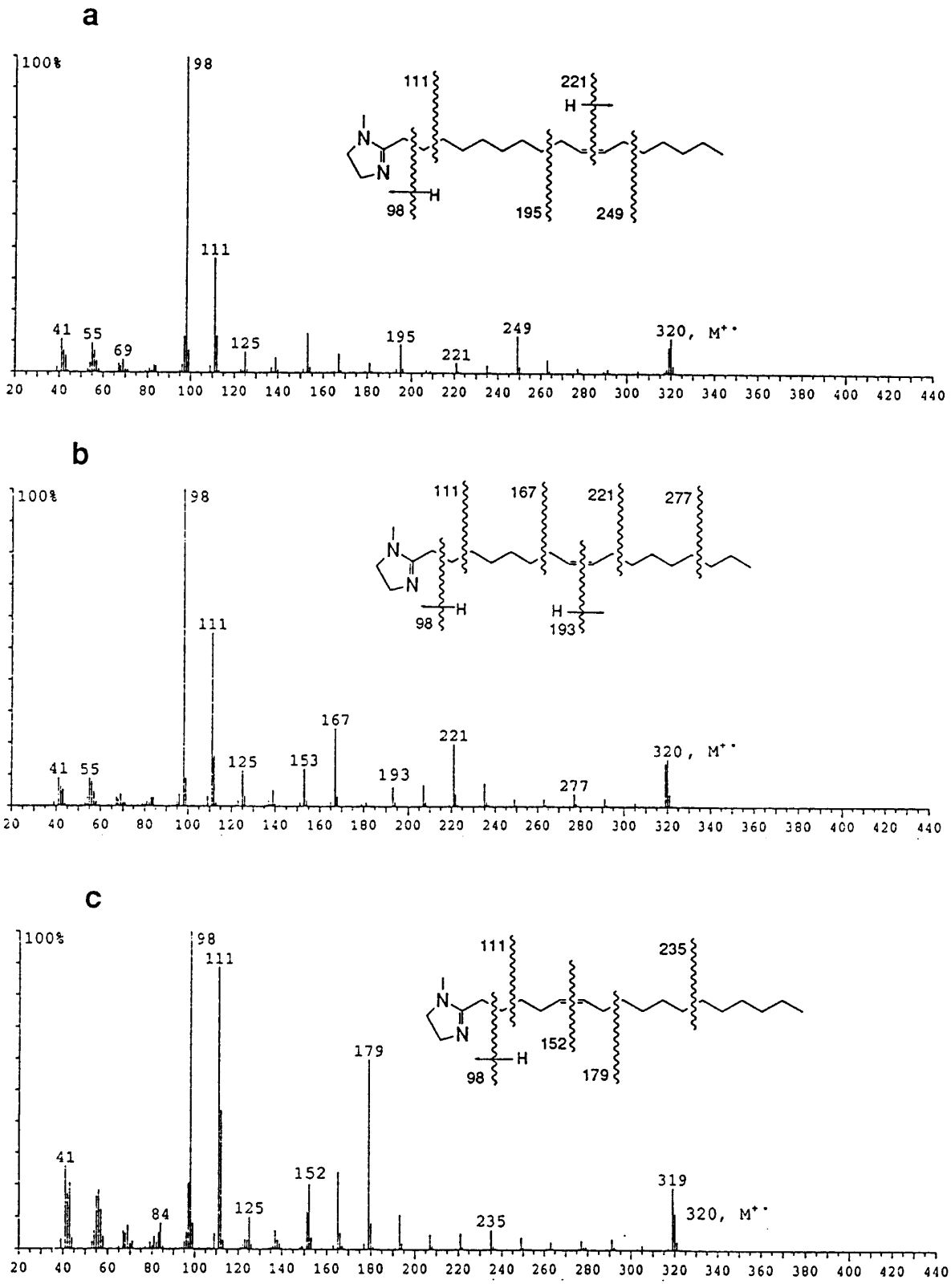


Figure 2. Electron impact mass spectra (70 eV) of the MIM derivatives of a variety of natural fatty acids: (a) vaccenic acid; (b) oleic acid; (c) petroselenic acid; (d) α-linolenic acid; (e) arachidonic acid; and (f) docosahexaenoic acid. The essential features of the fragmentations are indicated on the structures.

spectra of C_{18}–α,ω-dicarboxylic acid, differently derivatized on both ends, demonstrate the competing pathways of fragmentation. Figure 3(a) and (b) show that the influence of the ester group on fragmentation is almost completely suppressed by the MIM and the DMOX group, respectively, as indicated most conspicuously by the absence of peaks at *m/z* 74 and 87 which dominate the spectra of saturated fatty acid methyl esters. The spectrum in Fig. 3(c) shows that the DMOX and MIM groups have to compete in their influence on the frag-

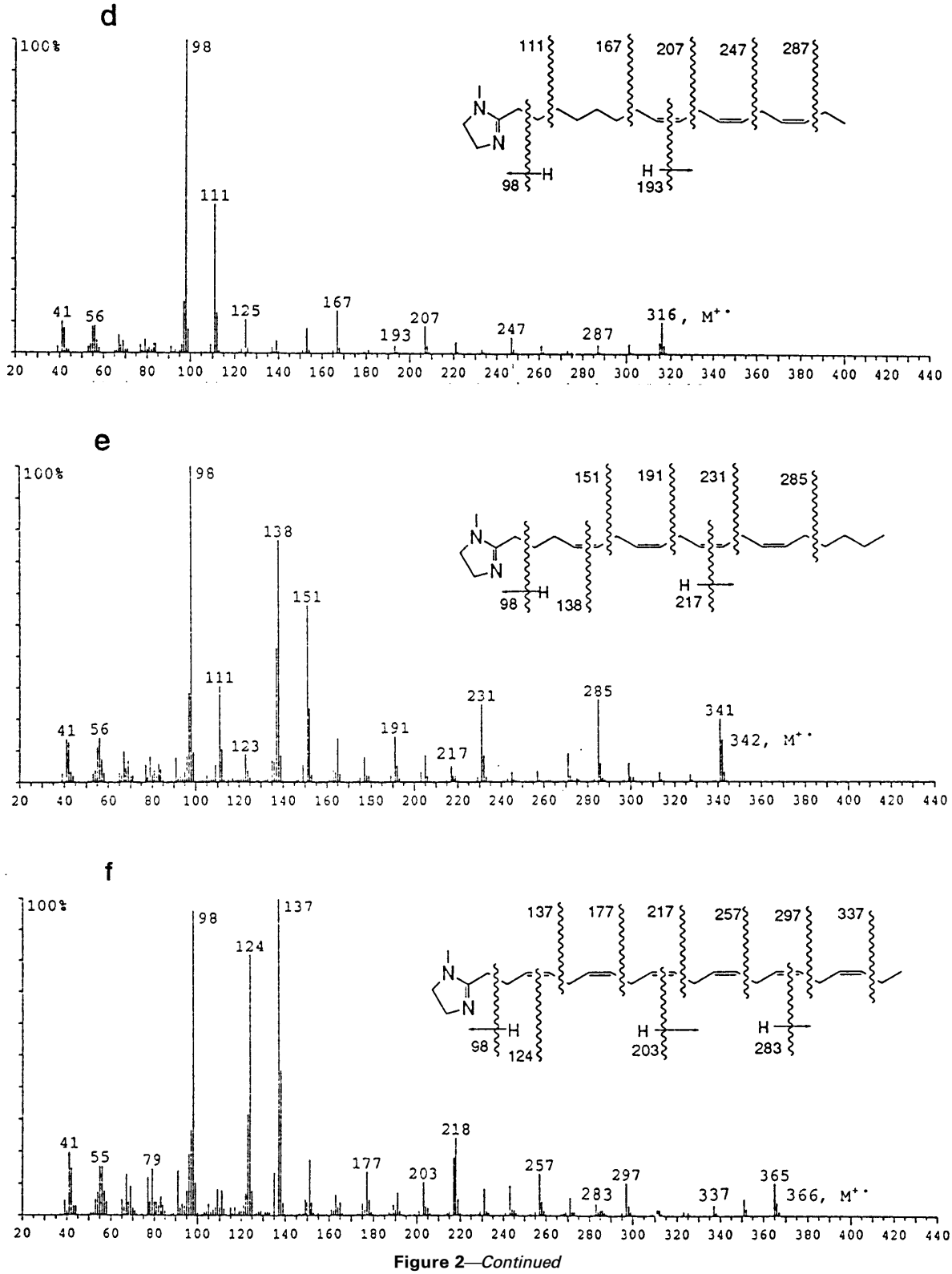


Figure 2—*Continued*

mentation. The MIM group is the clear winner, as indicated by the very low intensity of the peaks at *m/z* 113 and 126, typical of DMOX and thus dominant in Fig. 3(b). The suppressed groups are only apparent in the upper mass range by modification of the MIM-induced fragmentation, as seen in Fig. 3(b) and (c).

ESI-MS/MS of MIM derivatives

Figures 4–6 show the collision-induced dissociation (CID) mass spectra of the same set of compounds as before, but now from protonated molecules that are

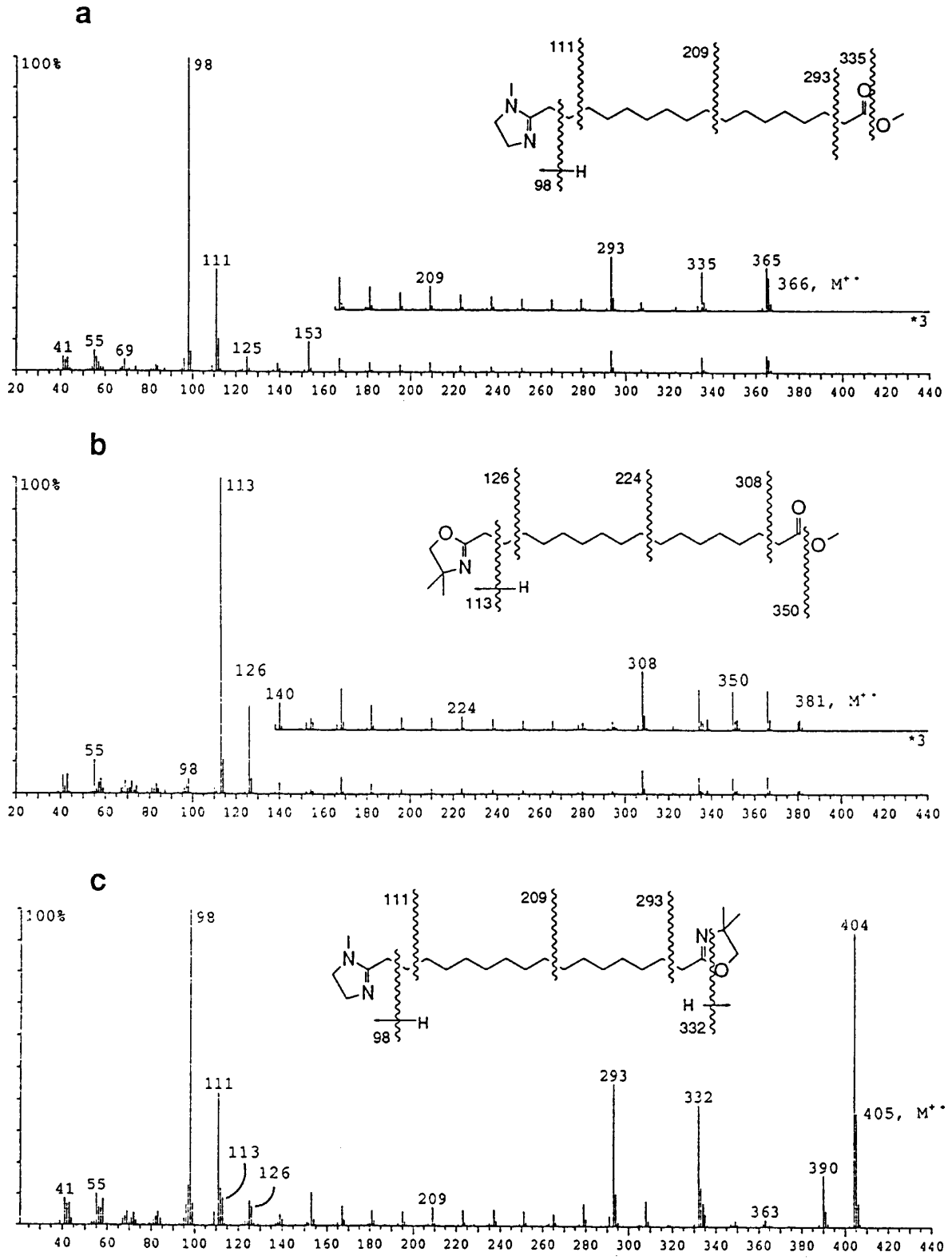


Figure 3. Electron impact mass spectra (70 eV) of three derivatives of C_{18}-α,ω-dicarboxylic acid: (a) α-MIM-ω-methyl ester derivative; (b) α-DMOX-ω-methyl ester derivative; and (c) α-MIM-ω-DMOX derivative. The essential features of the fragmentations are indicated on the structures.

generated by ESI. In contrast to the usual conditions for charge-remote fragmentation, where high collision energies are employed,[1–3] these experiments were conducted in a triple quadrupole instrument, where the collision energy was limited to 60 eV.

The results obtained from saturated chains [Fig. 4(a) and (b)] are very similar to those generated by EI, despite the different mechanisms at work in the two types of parent ions. An essentially radical mechanism, starting from the ionized functional group, as shown in

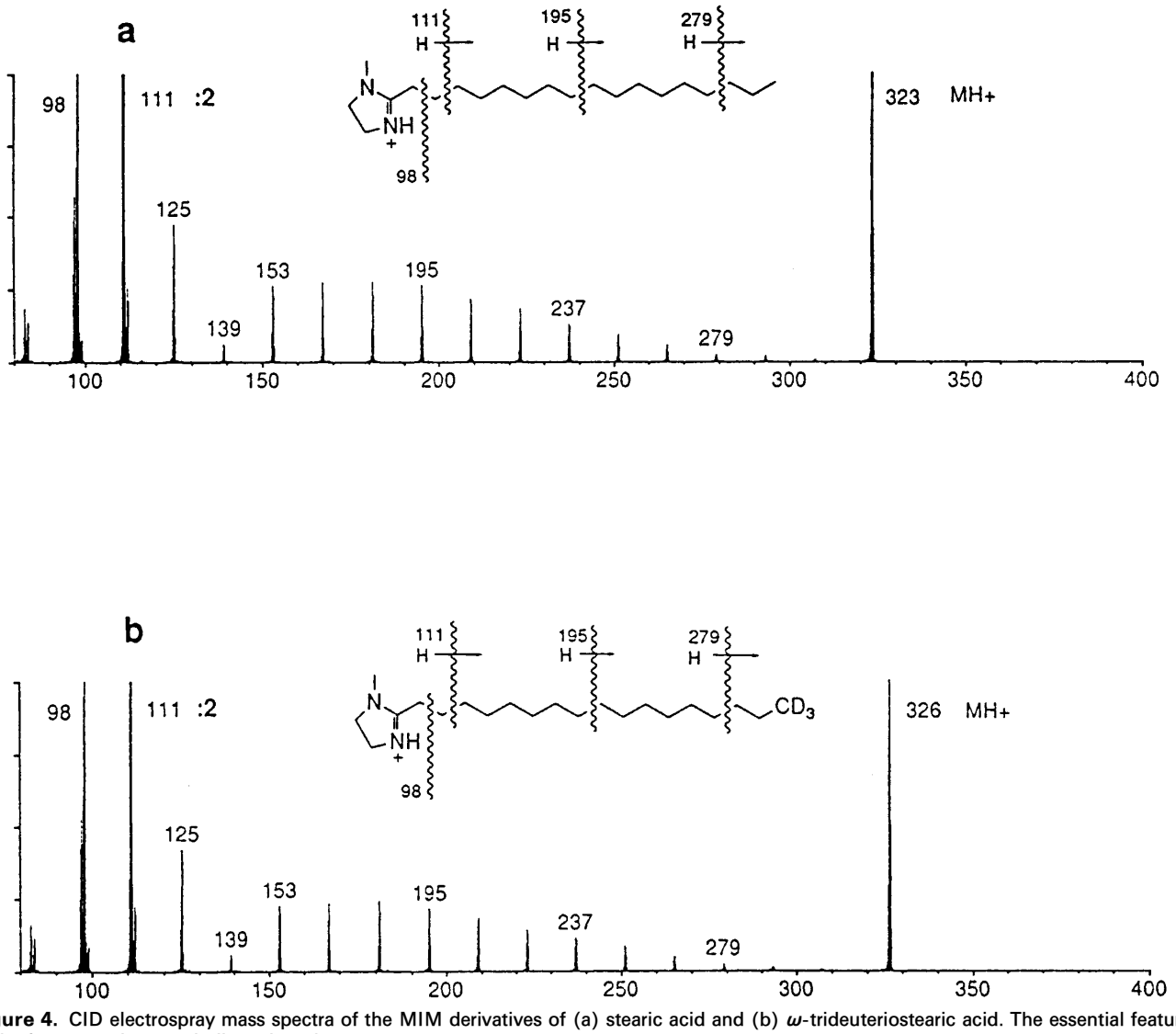


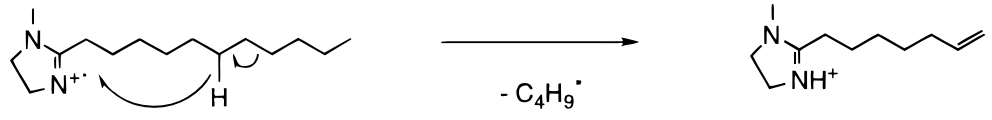
Figure 4. CID electrospray mass spectra of the MIM derivatives of (a) stearic acid and (b) ω-trideuteriostearic acid. The essential features of the fragmentations are indicated on the structures.

Scheme 2,[28,29] is operating in the radical ion obtained by electron impact. In contrast, a charge-remote electrocyclic reaction, shown in Scheme 3(a), has been proposed[10,12] as the main process occurring in the protonated ion that is generated by the ESI technique. Alternative charge-remote radical reactions, as shown in Scheme 3(b)[34,35] and (c),[36] which all lead to the same type of even-electron ions, may also be envisaged.

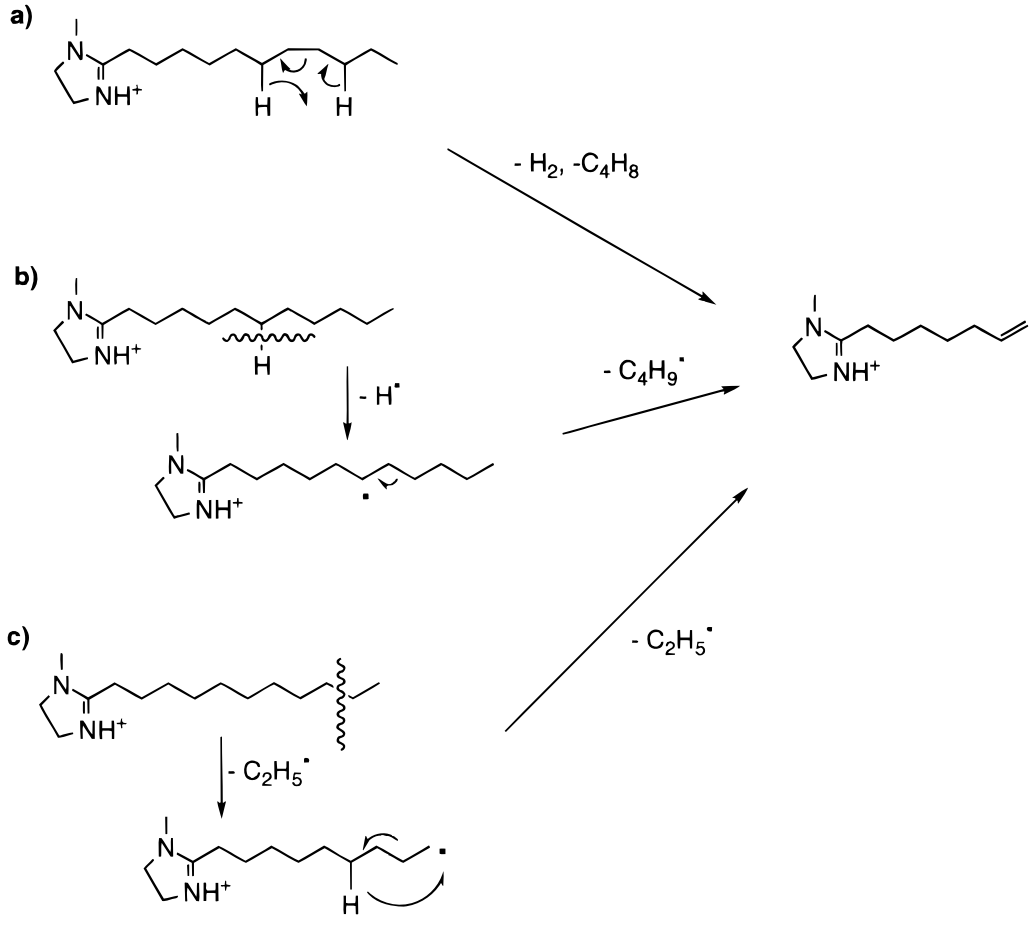
The radical ion at *m/z* 98 requires a radical cleavage of the chain.[9,19,20] It is remarkable that this odd-electron ion is formed here from a protonated, even-electron parent ion. Its structure is probably the same as that in the EI analogue, which is, however, formed by the McLafferty rearrangement from the (unprotonated) molecular radical ion.

Allowing for the overall similarity of the two types of spectra, a small but significant difference is nevertheless observed. Although all peaks for the derivative of stearic acid in the relevant mass range from *m/z* 98 upwards are at the same *m/z* values in both spectra [compare Figs 1(a) and 4(a)], a slight difference appears on comparing the spectra of the ω-D_3-stearic acid [compare Figs 1(b) and 4(b)]. Here the small peaks observed in the EI mass spectrum, due to the extrusion of ethyl and propyl radicals from the interior of the chain (marked with arrows in the EI mass spectrum) as discussed already, do not appear. This reflects the different mechanisms prevailing in the two types of spectra.

Comparing the ESI mass spectra of the unsaturated series [Fig. 5(a)–(f)] with the corresponding EI mass spectra, it is once more evident that most of the peaks occur at the same mass numbers. It is obvious, however, that the intensities of many peaks in the series, particularly in the upper mass range, are insufficient for complete structural analysis under the conditions employed (the intensities of the base peaks are halved in most of



Scheme 2. Basic mechanism for the chain fragmentation of radical molecular ions, obtained from the MIM derivative.



Scheme 3. Possible mechanisms for the charge-remote fragmentation of even-electron quasi-molecular ions, obtained from the protonated MIM derivative.

the figures). Increasing the collision energy within the low-energy range of a quadrupole instrument lowers the parent peak but does not drastically change the fragmentation pattern.

For vaccenic acid [Fig. 5(a)] and oleic acid [Fig. 5(b)], it is possible to recognize the position of the double bond. The cleavage of the allylic bond with loss of a hydrogen atom, as required by the charge-remote mechanisms shown in Scheme 3,[10,12,36–38] on the proximal side of the functional group, and to a lesser extent on the distal side, clearly defines its position: *m/z* 195 and 249 in vaccenic acid and *m/z* 167 and 221 in oleic acid. However, in these spectra the problem which obstructs the interpretation of the ESI mass spectra of the more highly unsaturated acid derivatives becomes apparent: there are satellite peaks two mass units above the distal allylic peaks, at *m/z* 251 [Fig. 5(a)] and *m/z* 223 [Fig. 5(b)]. In principle, they can have two causes. The first could be that the double bond has moved towards the far side of the functional group before cleavage. More likely, however, is the second possibility, which consists in a process which cleaves the allylic bond with a concomitant shift of a hydrogen atom to the double bond, so that the ion gains one mass unit instead of losing one. Thus the peak is always found 2 Da above the 'regular' peak. In other analogues with progressively increasing unsaturation this type of reaction becomes fairly frequent [Fig. 5(c)–(f)] and gives rise to ambiguities with regard to double bond localization. The most conspicuous case occurs in the spectrum of linoleic acid [Fig. 5(d)], where three strong peaks are marking prominent chain fragments. Two of the fragments, *m/z* 167 and 261, can arise by one of the mechanisms proposed in Scheme 3. However, the third, *m/z* 209, does not belong to this series. It has not lost but instead has *gained* a hydrogen atom in the course of the fragmentation.

In the spectrum of MIM-arachidonic acid [Fig. 5(e)], the most intense peaks of this type are found at *m/z* 153 and 193. In the next spectrum [Fig. 5(f)], such peaks are most evident at *m/z* 139, 179, 219 and 259. In most cases these peaks are accompanied by peaks attributed to the mechanisms proposed in Scheme 3, with varying intensity ratios. In contrast to the case of monoolefinic chains discussed above, it seems that in these methylene-separated polyolefins cleavage of the *vinylic* bond on the distal side of the functional group mostly results in fragments of this type. This rearrangement has been observed before and tentative mechanisms have been proposed.[14,34,35]

No matter which bond is broken, when combined with the proton added upon chemical ionization, this reaction always results in an upward shift of 2 Da relative to the analogue peak observed in the EI mass spectrum.

Fragmentation at double bond positions which are separated from the functional group by more than six carbons gives rise to relatively small peaks, mostly doublets with the usual distance of 2 Da. In contrast to the EI mass spectra, the missing intensity of these peaks

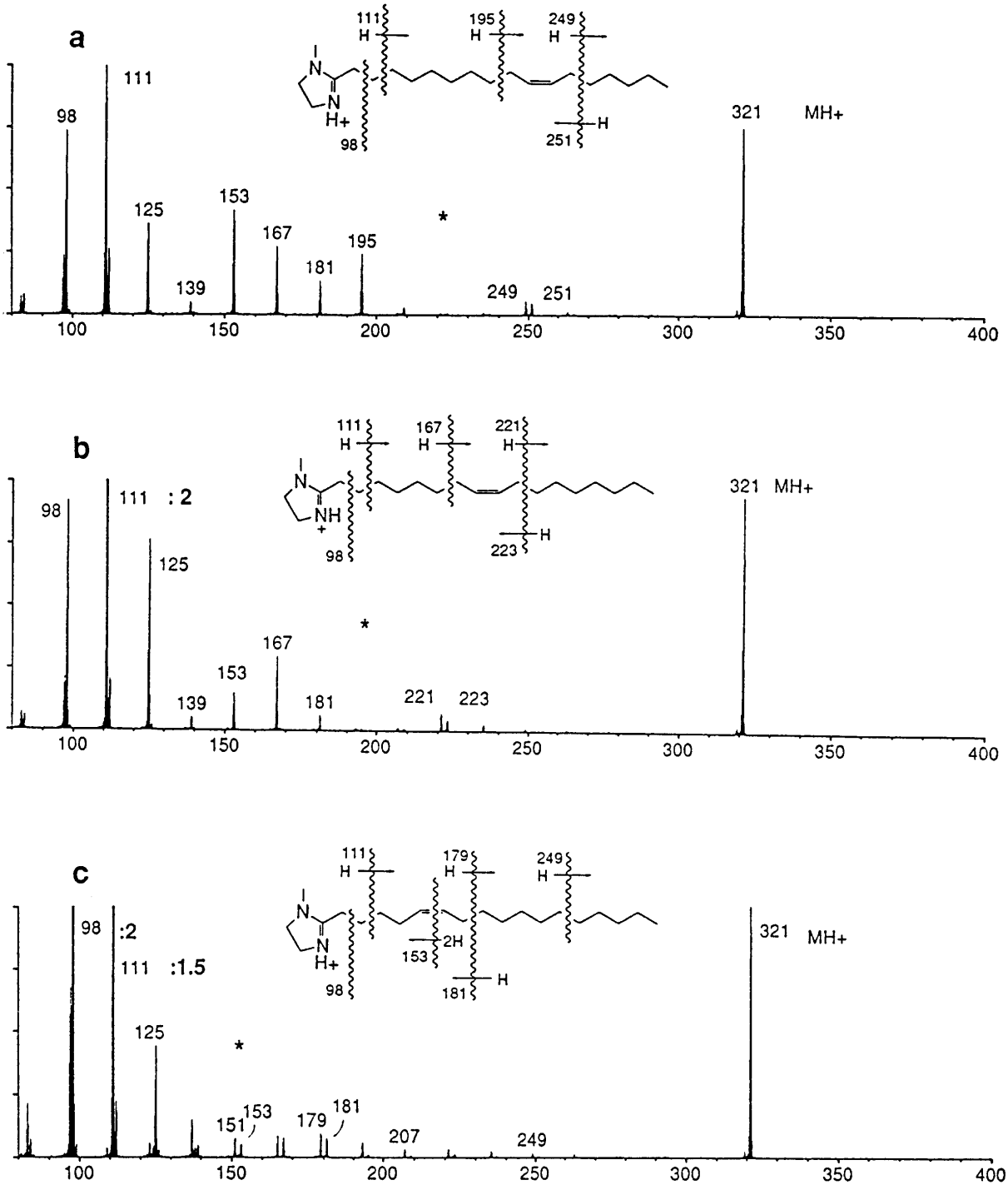


Figure 5. CID electrospray mass spectra of the MIM derivatives of a variety of unsaturated natural fatty acids: (a) vaccenic acid; (b) oleic acid; (c) petroselenic acid; (d) α-linolenic acid; (e) arachidonic acid; and (f) docosahexaenoic acid. The essential features of the fragmentations are indicated on the structures; the positions of the double bonds in the spectra are marked with asterisks.

can be regarded as an indication of the positions of the double bonds, marked by asterisks in Fig. 5(a)–(f).

The situation is different with respect to the cleavage of double bonds close to the functional group, e.g. at positions 4, 5 and 6, already discussed because of their peculiar fragmentation upon EI. In contrast to the cleavage observed in the EI mass spectra, the corresponding fragmentation in the present case formally represents a cut through the double bond with loss of *two* H atoms from the protonated fragment: *m/z* 151 [Fig. 5(c), double bond in position 6]; *m/z* 137 [Fig. 5(e), position 5]; *m/z* 123 [Fig. 5(f), position 4]. Again, a complicated mechanism, including a shift of the double bond towards the functional group, is presumably operating, which is not understood.

The present data do not permit us to formulate definitive rules for the fragmentation of the MIM derivatives of polyenic fatty acids. Thus these fragmentation

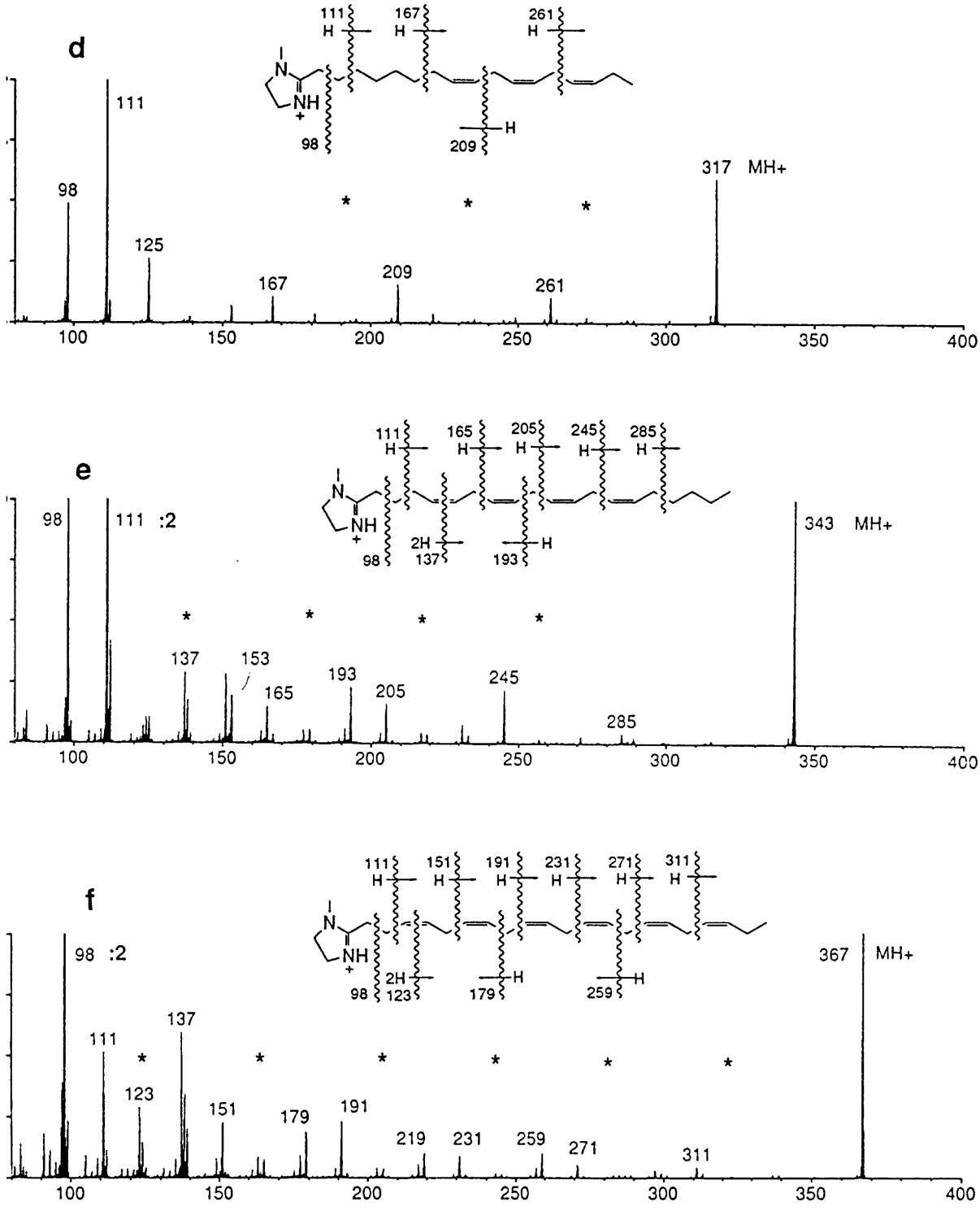


Figure 5—*Continued*

patterns at present constitute only fingerprints for these fatty acids. Studies with a greater variety of such compounds could well provide a basis for the interpretation of unknowns which is as reliable as that for the EI mass spectra of these derivatives.

As stated at the beginning, all experiments were carried out at low collision energies. Using the high-energy collision technique one could possibly simplify the fragmentation pattern by reducing the occurrence of competing rearrangements, and improve the completeness of the fragment series, as has been shown for other derivatives.[10,14,20]

Figure 6(a)–(c) compare the behaviour of the MIM and the DMOX derivatives in competition with the ester group and with each other upon protonation of the molecule. As expected, the MIM derivative wins the competition by a large margin also under these conditions. The strong basicity of this derivative ensures that the proton is placed on the amidine group, so that only fragments which carry this group appear in the spec-

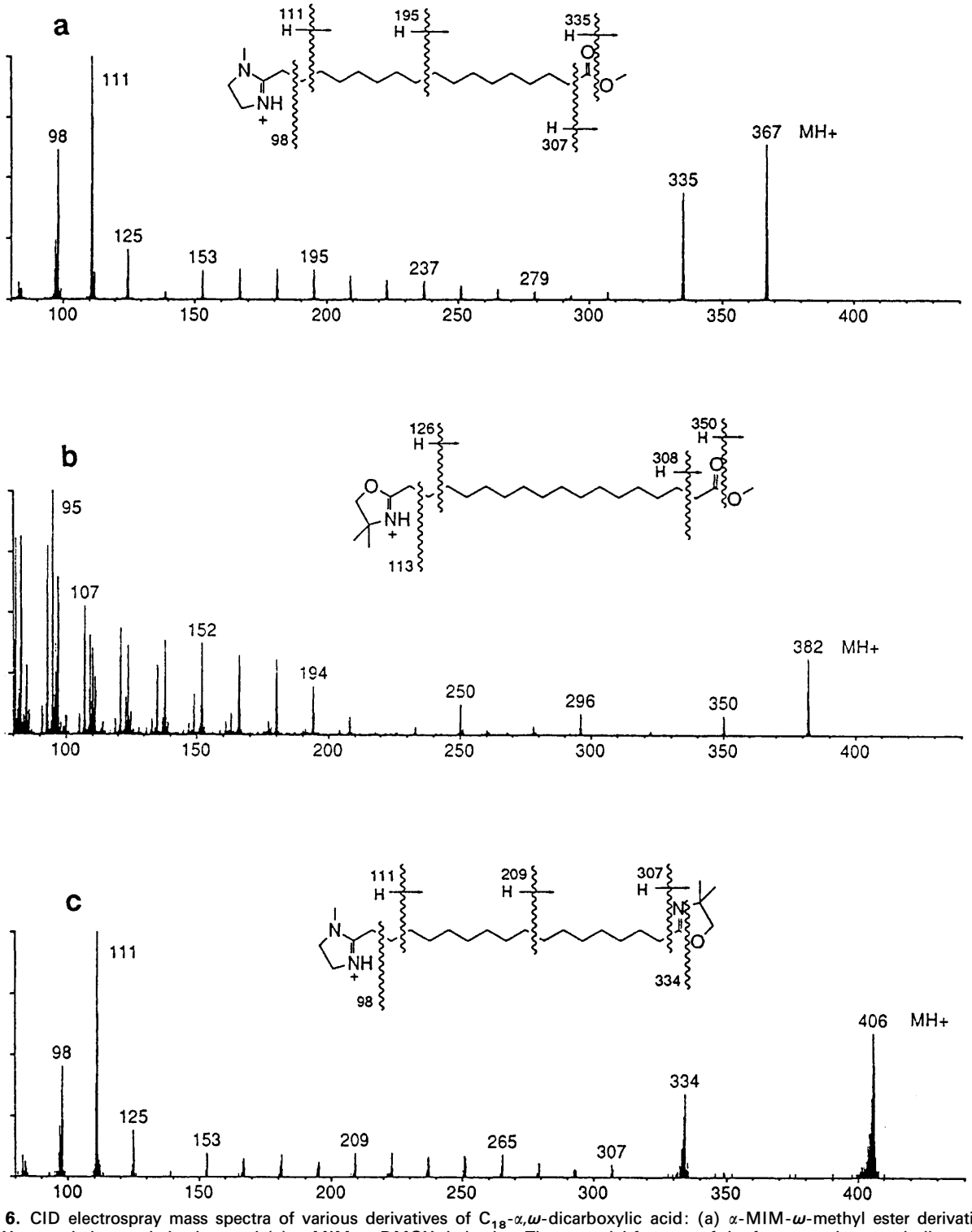


Figure 6. CID electrospray mass spectra of various derivatives of C_{18}-α,ω-dicarboxylic acid: (a) α-MIM-ω-methyl ester derivative; (b) α-DMOX-ω-methyl ester derivative; and (c) α-MIM-ω-DMOX derivative. The essential features of the fragmentations are indicated on the structures.

trum. The fragmentation of the chain is weak but corresponds closely to that of the stearic acid derivative, except for the uppermost mass range, where cleavage of the second functional group occurs, as indicated in the figures.

Surprisingly, the DMOX derivative does not induce the typical chain fragmentation under these conditions [Fig. 6(b)]. This difference in behaviour between the MIM and DMOX derivatives is presumably due to the strongly different stability of the two protonated species. In contrast to the MIM derivative, the DMOX derivative does not lock the charge, so that charge-induced fragmentation prevails,[21] from which structural features cannot easily be deduced.

CONCLUSIONS

The *N*-methylimidazoline derivative provides an improved possibility for studying structures of fatty acids upon electron impact and to a lesser extent upon electrospray ionization. Owing to its strongly basic character the derivative controls fragmentation in both types of ionization even in the cases where it has to compete with other functional groups, also known to control strongly the fragmentation of long chains, e.g. the dimethyloxazoline derivative.

Upon EI the localization of double bonds is possible even in highly unsaturated acids. Upon ESI at low collision energy the results are more modest because the series of fragment ions is incomplete in some cases and only partial localization of the double bonds is possible in the case of polyunsaturation. This is due to the occurrence of more than one fragmentation mechanism.

It would be interesting to study the protonated derivatives under the conditions of high-energy collisions.

As with all other derivatives of the carboxyl groups of unsaturated fatty acids, the fragmentation patterns show no significant difference between geometrical isomers and GC retention times have in addition to be used to resolve such problems.

Acknowledgements

The authors are grateful to Ms L. Allemann and A. Rippstein for performing the GC/MS analyses, to Dr W. Walther and Mr M. Noack and Mrs S. Franzke for developing the derivatization reactions and to Dr E. Kitas for help with the manuscript.

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