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Isotope-selective Tandem Mass Spectrometry: a New Tool for Elucidation of Fragmentation Pathways

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The isotopomeric ions of non-isotope-enriched biomolecules generated by nanoelectrospray ionization were selectively fragmented by collision-induced dissociation in a triple-quadrupole mass spectrometer. Using this technique, the influence of heavy isotopes, such as ^{13}C or ^{18}O, on the isotopic pattern of fragment ions is significantly enhanced compared with the dissociation of an entire group of non-mass selected molecular ions. Dissociation of the +1 Da isotopomeric ions results in fragment ion doublets, allowing the determination of the number of carbon atoms in the fragment when the empirical formula of the precursor molecular ion is known. Dissociation of the +2 Da isotopomeric ions results in fragment ion triplets. On the basis of the carbon atom number of a fragment, the ion abundances of these triplets allow the determination of a heteroelement such as oxygen, containing a stable isotope two mass units heavier than the value of its principle isotope. The technique was applied to the determination of the elemental composition of the two main fragment ions of protonated 1-alkenyl-2-acyl-phosphatidylethanolamine (plasmenyl-phosphatidylethanolamine). The results show that they are formed by a rearrangement resulting in two complementary fragment ions carrying the 1-alkenyl and 2-acyl substituent, respectively. Information on the elemental composition of fragment ions can be obtained by isotope-selective tandem electrospray mass spectrometry using sample amounts in the low picomole range. © **1998 John Wiley & Sons, Ltd.**

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KEYWORDS: tandem mass spectrometry; electrospray; stable isotope; elemental composition; plasmenyl-phosphatidylethanolamine

INTRODUCTION

The extraction of structural information from mass spectra is the basis for the widespread application of mass spectrometry in organic chemistry. Structural information was amenable from electron impact ionization spectra as fragmentation pathways could be established. For this task, accurate mass measurement of fragment ions and isotopic labelling of precursor ions were the most useful tools. Fragmentation pathways described so far mainly refer to the dissociation of molecular radical ions[1] as observed in electron impact ionization. Since the introduction of soft ionization techniques such as chemical ionization, fast atom bombardment or electrospray ionization (ESI) generating less excited even-electron ions, the fragmentation behaviour of these ions has been increasingly studied. In general, these even-electron ions require additional activation for efficient fragment ion formation, which normally is achieved by collision with atoms or molecules, which results in collision-induced dissociation (CID).[2] Although even-electron molecular ions frequently dissociate by a single heterolytic bond cleavage,[3] a broad variety of more complex fragmentation reactions of even-electron ions is known[3–5] which are characterized by multiple bond cleavages and rearrangement reactions. Another problem with respect to fragment ion identification is the discrimination between isobaric fragment ions such as $[HSO_4]^-$ and $[H_2PO_4]^-$ ions.[6] Hence data on the isotopic composition of fragment ions of even-electron molecular ions are often very helpful or even necessary for their correct assignment. In the following, we extend the description of isotope-selective tandem mass spectrometry (MS/MS)[6] using a triple-quadrupole instrument and ESI to the determination of the carbon and oxygen content of fragment ions. In addition, application of this method is demonstrated for the structure elucidation of two so far unidentified fragment ions occurring in the positive ion electrospray product ion spectra of 1-alkenyl-2-acyl-phosphatidylethanolamine (plasmenyl-phosphatidylethanolamine).[7] Determination of their elemental composition was used as basis to propose a fragmentation mechanism.

EXPERIMENTAL

Myristoylcarnitine and phosphatidylethanolamine (type I, from bovine brain) were obtained from Sigma

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(Deisenhofen, Germany). Solutions of these compounds in chloroform–methanol (2:1 v/v) at a concentration of about 30 pmol μl^{-1} were used for MS analysis. For the analysis of plasmenyl-phosphatidylethanolamine the solution was acidified with 1% acetic acid.

Mass spectra were acquired using a TSQ 7000 triple-quadrupole instrument (Finnigan MAT, San Jose, CA, USA) equipped with a nano-electrospray ion source (EMBL, Heidelberg, Germany) operating at a flow rate of 20–50 nl min^{-1}. The orifice of the electrospray capillary was positioned at about a 1 mm distance directly in front of the heated transfer capillary held at 150 °C. The needle voltage was +400 to +700 V. Argon was used as the collision gas at a pressure of 2 mTorr (1 Torr = 133.3 Pa). For determination of the isotopic fragment ion abundances a mass range of about 6 Da was scanned using a scan time of 3 s per scan. Sets of 40 scans were averaged for a single data point, and three data points obtained in this way were used to calculate an arithmetic mean and standard deviation.

RESULTS AND DISCUSSION

Fragmentation of +1 Da isotopomeric ions of a molecular ion cluster

The most important difference in the isotopic composition of the monoisotopic ion and the first isotope peak in a molecular ion group is the presence of one atom of ^{13}C in the first isotope peak. When nitrogen is present in the molecule, a certain fraction of the first isotope peak contains one atom of ^{15}N. Owing to the lower natural abundance of ^{15}N compared with ^{13}C (0.3% *vs.* 1.1%) and to the lower abundance of nitrogen compared with carbon in most organic compounds, the contribution of ^{15}N is neglected in the following calculations. When the +1 Da isotopomeric ions of a molecular ion group dissociates, the ^{13}C atom is distributed at random between the fragment ion and the neutral fragment according to the ratio of carbon in these fragments. The calculation of the ion abundances is shown in Table 1 for the case of a molecular ion of composition $^{12}C_{20}\,^{13}C_1$ which dissociates into a C_4 fragment ion and a C_{17} neutral fragment.

When the number of carbon atoms in the molecular ion is known, the number of carbon atoms in the fragment ion can be measured in this way. Fragmenting the +1 Da isotopomer of a molecule enhances the influence of the carbon number in the fragment ion on its $^{13}C/^{12}C$ ratio compared with the fragmentation of an unselected molecular ion group, as performed by skimmer CID, for example. The dependence of the $^{13}C/^{12}C$ ratio on the number size (in C atoms) of the fragment ions was calculated for the fragmentation of a molecular ion containing 21 carbon atoms, both for the unselected molecular ion group as precursor and for the first isotopomeric ion as precursor. The results are shown in Fig. 1.

Without isotope selection in the molecular ion group, each additional ^{13}C atom in the fragment ion contributes to the first isotope peak in the normal way with about 1.1% relative abundance. However, by selecting the +1 Da isotopomer of a molecular ion group as precursor, the contribution of a single carbon atom to the isotope pattern of a fragment ion is strongly enhanced, facilitating the determination of the carbon number in a fragment ion. As demonstrated by the data in Fig. 1, the set of possible values of the $^{13}C/^{12}C$ ratio in the fragment ion can be calculated exactly when the number of carbon atoms in the molecular ion is known. The data calculated in this way are valid both for isotopically non-enriched and for enriched sample molecules; they do not depend on the exact ^{13}C abundance of the precursor molecule investigated, since each molecule in the investigated subset contains exactly one ^{13}C atom. The effect is demonstrated for the fragmentation of myristoylcarnitine, a C_{21} biomolecule the ESI spectrum of which shows a molecular ion at *m/z* 372.3 (monoisotopic value). The skimmer CID product ion spectrum in Fig. 2 shows the presence of five fragment ions at *m/z* 60, 85, 144, 211 and 313.

The ion doublets of these fragment ions generated from the +1 Da isotopomeric ion at *m/z* 373.3 as precursor ion and the proposed structures of the fragment ions are displayed in Fig. 3. As expected, the relative abundance of the ^{13}C isotope peak increases with increasing carbon number of the fragment ion. The experimental accuracies are sufficient for the determination of the carbon contents of the fragment ions, as shown by the isotopic abundance levels calculated for the presence of one carbon atom more or less in the fragment ions. The main source of error in these measurements is the spillover of adjacent peaks due to insufficient resolution at the stage of precursor ion or product ion analysis. Therefore, baseline resolution should be established at both stages of mass analysis to obtain reliable isotope abundance data. An additional error may arise from instrumental characteristics such as mass-dependent ion transmission and mass-dependent detection efficiency.

Table 1. Calculation of the isotopic abundances in a fragment ion doublet generated by CID of the first $^{13}C_1$-containing isotopomers in a molecular ion cluster[a]

$^{12}C_{20}\,^{13}C_1$ molecular ion		No. of	Relative abundances in
C_{17} neutral fragment	C_4 ion	structures	C_4 fragment ion (%)
^{13}C		17	80.95
	^{13}C	4	19.05

[a] The example is calculated for a $^{12}C_{20}\,^{13}C_1$ molecular ion dissociating into a C_4 fragment ion and a C_{17} neutral fragment. A ^{13}C isotope peak of 19% relative abundance is observed.

Fragmentation of +2 Da isotopomeric ions of a molecular ion cluster

In biomolecules containing only C, H, N and O, the main components of the second isotope peak in molecular ion clusters correspond to isotopomers containing two atoms of ^{13}C or one atom of ^{18}O. For instance, neglecting the minor influences of ^{2}H and ^{15}N, the molecular ion composition of the second isotope peak

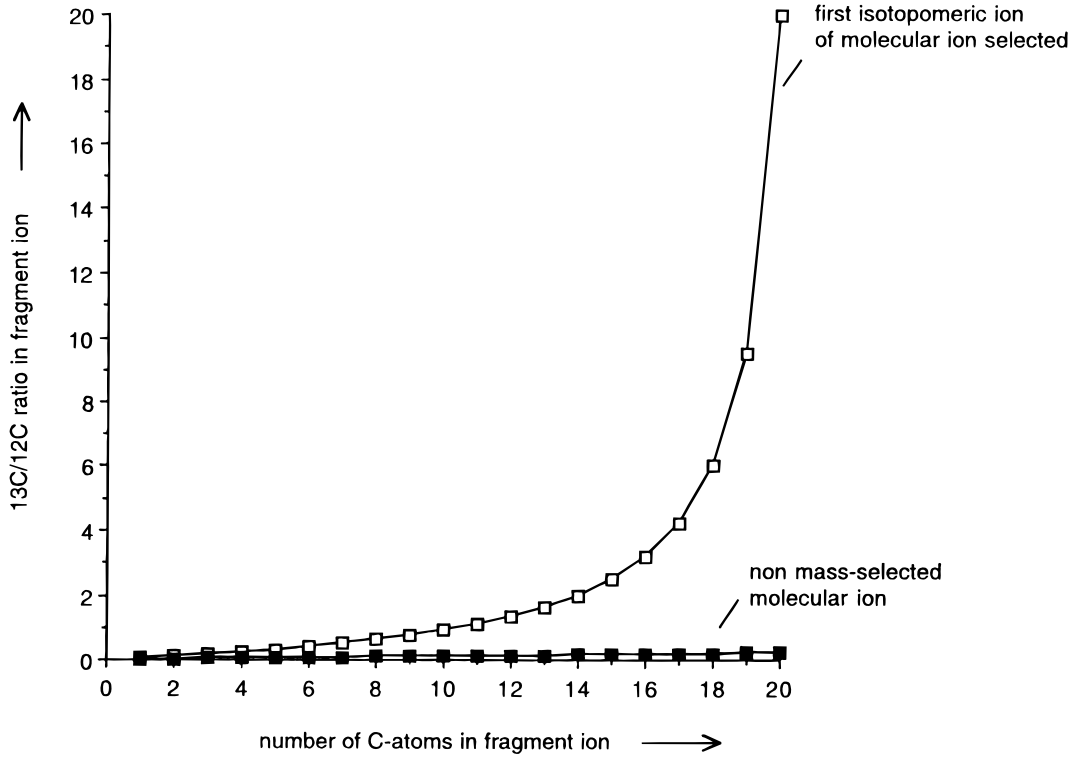

Figure 1. Calculated intensity ratio of the $[^{13}C_1]$peak/$[^{13}C_0]$ peak of fragment ions originating from a C_{21} compound. The intensity ratio is plotted *vs*. the number of carbon atoms present in the fragment ion (i) for CID of the molecular ion of the C_{21} compound without isotope selection (filled symbols) and (ii) for CID of the $[^{13}C_1]$-isotopomer of the molecular ion of the C_{21} compound (open symbols). The influence of the fragment ion carbon number on the isotopic pattern of the fragment ion is markedly enhanced using isotope-selective CID.

of the myristoylcarnitine ion at *m/z* 374.2 is calculated to be 76.1% $^{12}C_{19}\,^{13}C_2\,^{16}O_4$ and 23.9% $^{12}C_{21}\,^{16}O_3\,^{18}O$. This approximate calculation was performed by comparing the second isotope peak abundances of the empirical formulae C_{21} and $C_{21}O_4$.

Fragmentation of the mass-selected +2 Da isotopomer (*m/z* 374.2) results in fragment ions showing signal triplets, indicating the absence of $^{13}C/^{18}O$, the presence of $^{13}C_1$ only or the presence of $^{13}C_2/^{18}O_1$. The resulting fragment ion triplets for the five fragment ions

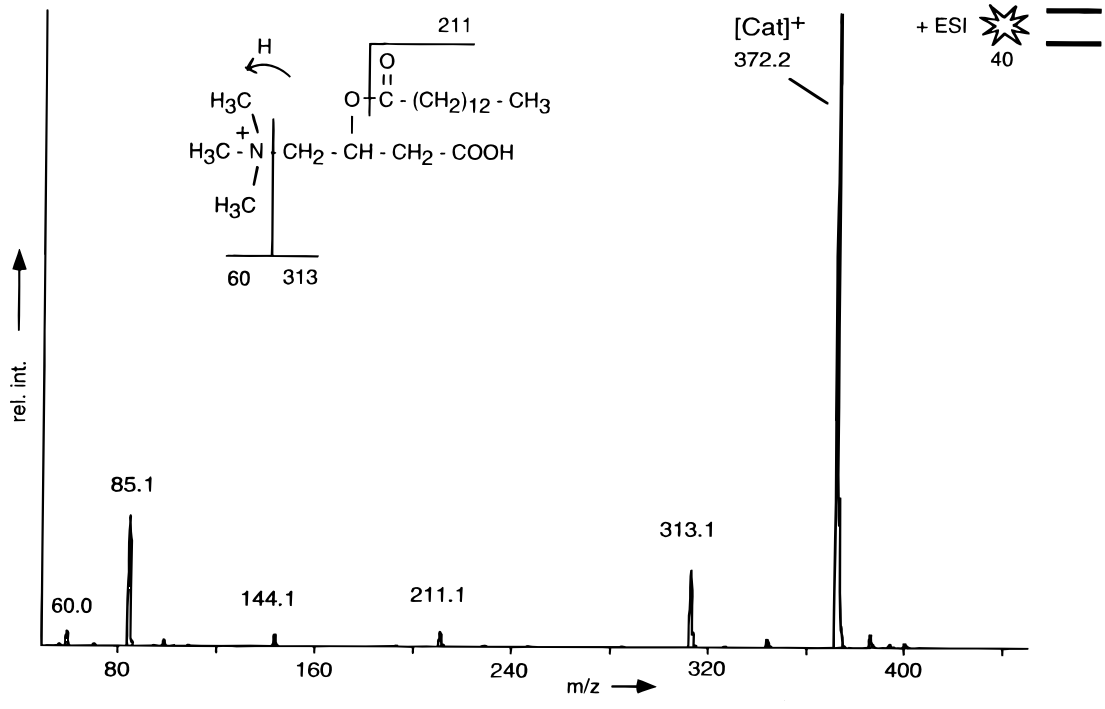

Figure 2. Positive ion electrospray product ion spectrum of myristoylcarnitine generated by skimmer CID. Five fragment ions are observed at *m/z* 60, 85, 144, 211 and 313. Pictogram inserts are used as outlined in Ref. 8.

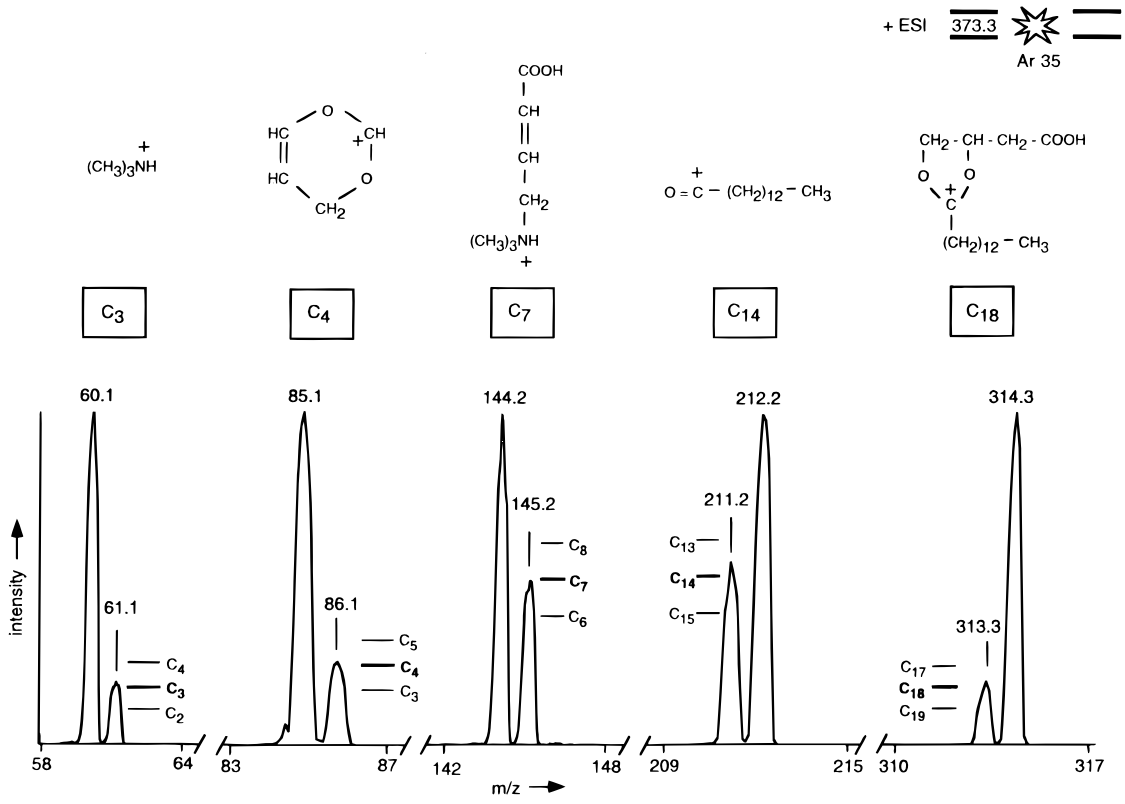

Figure 3. Isotope patterns of the myristoylcarnitine fragment ions displayed in Fig. 3 as observed by selecting the +1 Da isotopomer at *m/z* 373.3 as precursor ion. The isotope patterns of the fragment ions allow a direct readout of the carbon number present in the fragment ions.

of myristoylcarnitine when the ion signal at *m/z* 374.2 is selected as precursor ion are given in Fig. 4.

It is evident that the abundances of the heavy ions increase in proportion to the number of carbon atoms. When the carbon number in the fragment ion is known, e.g. by the analysis given in Fig. 3, the contribution of the ^{13}C atoms to these ion triplets can be calculated. This is done by calculating all possible positions for the two ^{13}C atoms, which include three situations: (i) both ^{13}C atoms occur in the neutral fragment, (ii) one ^{13}C atoms is present in the neutral fragment and the other in the fragment ion and (iii) both ^{13}C atoms are found in the fragment ion. The corresponding calculation is exemplified in Table 2 for the myristoylcaritine frag-

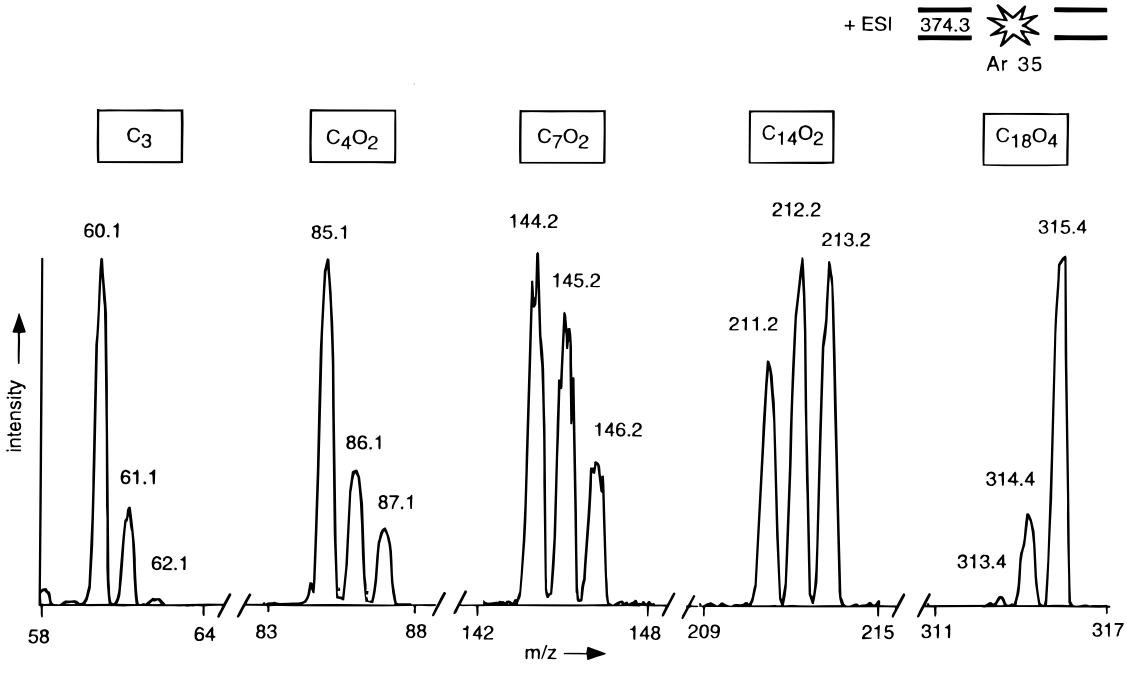

Figure 4. Isotope patterns of the fragment ions of myristoylcarnitine as observed by selecting the +2 Da isotopomer at *m/z* 374.3 as precursor ion. The isotopic abundances are influenced in particular by the presence of carbon and oxygen.

Table 2. Calculation of the isotope distribution in the fragment ions $C_4H_5O_2^+$ at *m/z* 85–87 generated by selective fragmentation of the +2 Da isotope ion of myristoylcarnitine at *m/z* 374.3[a]

	$C_{19}{}^{13}C_2{}^{16}O_4$ = 76.1% of second isotope peak				$^{12}C_{21}{}^{16}O_3{}^{18}O$ = 23.9% of second isotope peak		
C_{17} neutral fragment	$^{12}C_4$ ion	No. of structures	Abundance in C_4 fragment ion (%)	O_2 neutral fragment	O_2 ion	No. of structures	Abundance in C_4 fragment ion (%)
$^{13}C_2$		$\frac{17!}{2!(17-2)!}=136$	64.76	^{18}O		2	50
^{13}C	^{13}C	$4 \times 17 = 68$	32.38				
	$^{13}C_2$	$\frac{4!}{2!(4-2)!}=6$	2.86		^{18}O	2	50

Merging the contribution of carbon and oxygen

m/z	Contribution of C %		Contribution of O (%)	Sum (%)
85	(64.76 × 0.761)	+	(50 × 0.239)	61.23
86	(32.38 × 0.761)			24.64
87	(2.86 × 0.761)	+	(50 × 0.239)	14.13

[a] Based on the carbon number in the fragment determined as shown in Fig. 3, the influence of two ^{13}C isotopes is calculated. Then, the distribution of one ^{18}O atom between two positions in the fragment ion and two positions in the neutral fragment is calculated. Finally, both results are merged according to the isotopic composition of the +2 isotopomer of the molecular ion, i.e. 76.1% $^{12}C_{19}{}^{13}C_2{}^{16}O_4$ and 23.9% $^{12}C_{21}{}^{16}O_3{}^{18}O$.

ment ion at *m/z* 85, containing four carbon atoms out of 21 in the precursor molecular ion.

In addition to the influence of carbon, the presence of oxygen has to be taken into account, since the second isotope peak of myristoylcarnitine is generated by about 24% of molecular ions bearing one atom of ^{18}O. Dissociation of this subset of isotopomers results either in fragment ions containing only ^{12}C and ^{16}O (in case the ^{18}O atom is lost) or in +2 Da isotopomers of the fragment ions (in case the ^{18}O is retained). In analogy with the calculation in Table 1, this ratio depends on the number of oxygen atoms in the precursor and in the fragment ion. The contributions of ^{18}O and the merging of the contributions of ^{13}C and ^{18}O on the fragment ion triplet abundances are also exemplified in Table 2. When this type of calculation is performed for all five fragment ions of myristoylcarnitine, good agreement between the observed and calculated data is obtained. The corresponding data are summarized in Table 3.

The fragment ion triplet abundances are significant for the determination of the number of oxygen atoms in the fragment ion, since a variation of the oxygen atom number by one effects a variation in the ion abundance data which exceeds the experimental error. This is demonstrated in Table 4 for the fragment ion at *m/z* 85. The data show that this fragment ion contains two atoms of oxygen, a finding that supports the empirical formula of the proposed fragment ion structure given in Fig. 3.

Fragment ions of plasmenyl-phosphatidylethanolamine

We have been investigating the fragmentation behaviour of molecular ions of polar lipids for their structural characterization and the quantification of selected polar lipids and phospholipid classes in crude lipid extracts by MS/MS.[6,9]

Plasmenyl-phospholipids deserve special interest among the membrane phospholipids since they represent an important factor in the protection of cells against lipid peroxidation.[10,11] For instance, specific detection of phosphatidylethanolamine (PE) including plasmenyl-phosphatidylethanolamine from an unpro-

Table 3. Experimental and calculated data for the isotope abundances of the fragment ions as observed by CID of the +2 Da isotopomer of the myristoylcarnitine molecular ion group at *m/z* 374.2[a]

Ions		Monoisotopic ion abundance (%)	+1 Da isotopomer abundance (%)	+2 Da isotopomer abundance (%)
m/z 59–61	Expt.	0.7660 ± 0.006	0.2196 ± 0.0034	0.0144 ± 0.0026
	Calc. (C_3O_0)	0.7935	0.1956	0.0109
m/z 85–87	Expt.	0.6186 ± 0.0120	0.2419 ± 0.0104	0.1395 ± 0.0089
	Calc. (C_4O_2)	0.6123	0.2464	0.1413
m/z 144–146	Expt.	0.4518 ± 0.0031	0.3550 ± 0.0119	0.1932 ± 0.010
	Calc. (C_7O_2)	0.4492	0.3552	0.1956
m/z 211–213	Expt.	0.2656 ± 0.0144	0.3710 ± 0.0090	0.3633 ± 0.0122
	Calc. ($C_{14}O$)	0.2554	0.3552	0.3894
m/z 313–315	Expt.	0.0181 ± 0.0020	0.1880 ± 0.0097	0.7939 ± 0.00112
	Calc. ($C_{18}O_4$)	0.0109	0.1957	0.7934

[a] Data were calculated by taking into account only ^{13}C and ^{18}O data.

Table 4. Experimental isotopic abundances of the ions $C_4H_5O_2^+$ at *m/z* 85–87 containing four carbon atoms, as generated by CID of the +2 Da isotopomer of the molecular ion group of myristoylcarnitine at *m/z* 374.2[a]

Species	*m/z* 85 monoisotopic ion abundance (%)	*m/z* 86 +1 Da isotopomer abundance (%)	*m/z* 87 +2 Da isotopomer abundance (%)
Fragment ion C_4O_2 (measured)	0.6186	0.2419	0.1395
C_4O (calculated)	0.6720	0.2464	0.0816
C_4O_2 (calculated)	0.6123	0.2464	0.1413
C_4O_3 (calculated)	0.5526	0.2464	0.2010

[a] The data are compared with those calculated for the presence of one, two and three oxygen atoms showing that the fragment ion contains two of the four oxygen atoms present in the precursor molecular ion.

cessed total lipid extract can be achieved by scanning for neutral loss of 141 Da,[9] corresponding to expulsion of the polar head group, phosphoethanolamine. However, it was observed that the scan for neutral loss of 141 Da is more sensitive for diacyl-PE than plasmenyl-PE.[9] Therefore, the fragmentation behaviour of diacyl-PE and plasmenyl-PE was investigated under identical conditions and a less abundant $[M + H - 141]^+$ signal for plasmenyl-PE compared with diacyl-PE was observed, a finding that is in agreement with the above-mentioned discrimination. In addition to the $[M + H - 141]^+$ fragment of minor abundance, plasmenyl-PE shows a set of highly abundant fragment ions in the *m/z* region at about half the molecular mass. This is demonstrated in Fig. 5, which shows the product ion spectrum of a particular protonated molecular species of plasmenyl-PE at *m/z* 702.5. This ion was selected from the set of molecular ions observed by analysing a mixture of phosphatidylethanolamine from bovine brain containing about 60% of 1-alkenyl-2-acyl-phosphatidylethanolamine.

The two abundant fragment ions at *m/z* 339 and 364 cannot be explained by a simple bond cleavage. To obtain additional information on these fragment ions, we determined the number of carbon and oxygen atoms in these fragments by selective fragmentation of the +1

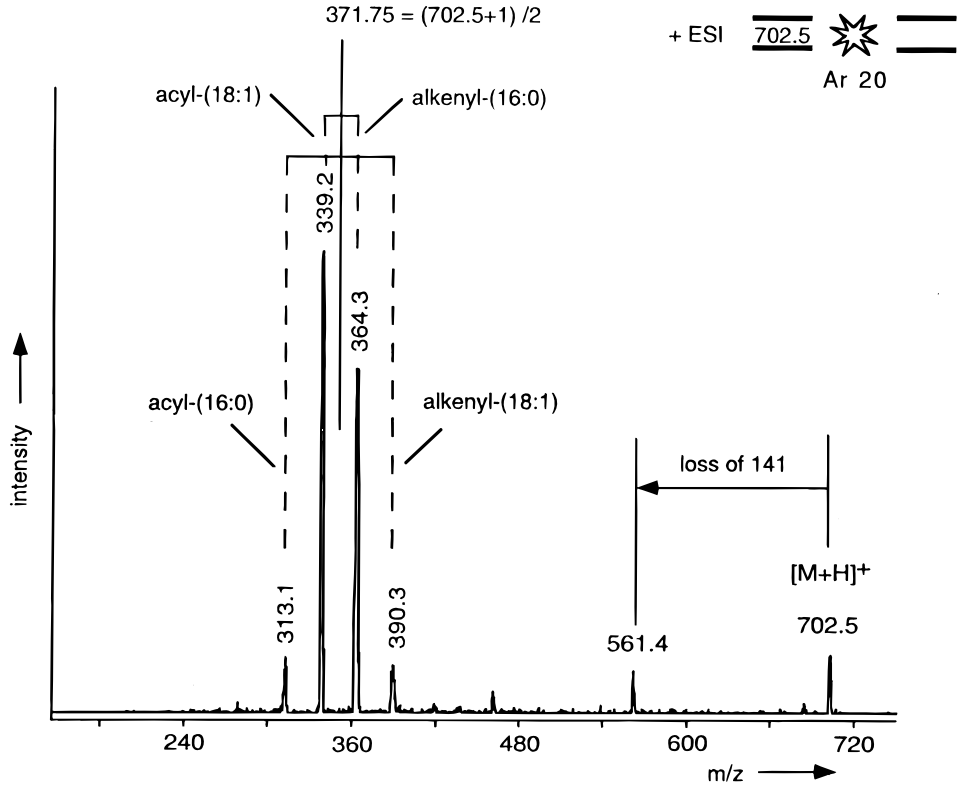

Figure 5. Positive ion ESI product ion spectrum of the ion at *m/z* 702.5 observed in the ESI spectrum of phosphatidylethanolamine from bovine brain. The ion represents the $[M + H]^+$ ion of plasmenyl-PE(34:1); (34:1) designates a molecular species containing substituents with a total of 34 carbon atoms and one extra double bond in the chain attached to either position 1 or 2 of the glycerol backbone. Complementary fragment ions generated by the mechanism outlined in Fig. 7 are distributed symmetrically around *m/z* (702.5 + 1)/2 = *m/z* 371.75.

Da and +2 Da isotopomers of plasmenyl-PE(34:1) at *m/z* 703.5 and 704.5, respectively. The experimental results obtained are given in Fig. 6.

The isotope patterns obtained by CID of the +1 Da isotopomer of plasmenyl-PE(34:1) (see Fig. 6, top) show that the fragments at *m/z* 339 and 364 contain 21 and 18 carbon atoms, respectively, since for this combination the best agreement between experimental and calculated data is obtained. On this basis, the isotope patterns observed by fragmentation of the +2 Da isotopomers (see Fig. 6, bottom) provide the information on the number of oxygen atoms present in the fragment ions. As can be inferred from Fig. 6, fluctuations in the peak shape limit the experimental accuracy. However, with regard to all abundance data, the minimal deviation between experimental and calculated data is clearly observed for the compositions $^{12}C_{21}{}^{16}O_3$ for the ion at *m/z* 339 and $^{12}C_{18}{}^{16}O_4$ for the ion at *m/z* 364. Since the heavier fragment ion at *m/z* 364 contains three carbon atoms less than the lighter fragment and

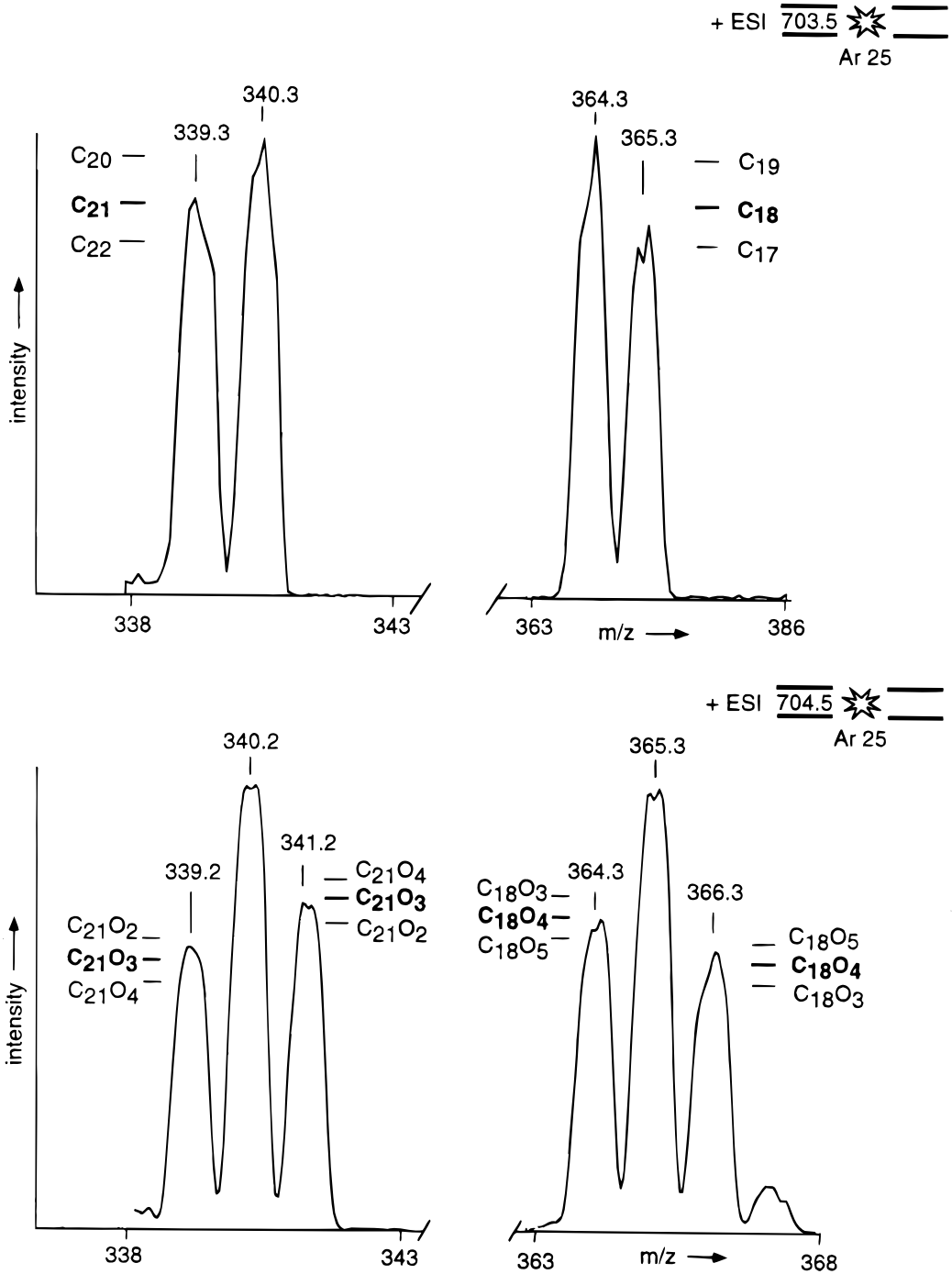

Figure 6. Positive ion ESI isotope patterns observed for the two abundant fragment ions in Fig. 5. (a) +1 Da isotopomers of plasmenyl-PE(34:1) $[M+H]^+$ ions at *m/z* 703.5 selected as precursor ion; (b) +2 Da isotopomers of plasmenyl-PE(34:1) $[M+H]^+$ ions at *m/z* 704.5 selected as precursor ion. The elemental compositions printed in bold provide the best agreement calculated and experimental data.

four oxygen atoms, it can be concluded that this fragment contains the phosphate group. The sum of the monoisotopic fragment ion m/z values is 703.5, which points to a complementary nature of these fragment ions. The proton that carries the positive charge is attached alternatively to one of the two fragments, so that both products of this fragmentation pathway are observed as ions. The elemental composition data support this interpretation, since the empirical formula of plasmenyl-PE(34 : 1) is $C_{39}H_{76}O_7NP$.

Based on this information, the fragmentation pathway shown in Fig. 7 is proposed, suggesting a transfer of the alkenyl group to the amino function with subsequent cleavage of the glycerol-C(3)–oxygen bond as the reaction mechanism.

For the initial structure, protonation at the alkenyl double bond is assumed. In the first step, a nucleophilic attack of the amino function at C(1) of the alkenyl chain is assumed, leading to an *N*-alkyl-Schiff base structure and a free hydroxy group at glycerol-C(1). In the second step, the phosphate group at glycerol-C(3) is cleaved off, a cleavage that is always accompanied by a proton transfer to the phosphate group. The origin of this proton determines which fragment is ionic or neutral. In the fragmentation route on the right-hand side of Fig. 7 the transferred proton stems from the protonated Schiff base structure. In this case the 2-acylglycerol fragment carries the charge. In the alternative route on the left-hand side the proton originates from the glycerol part so that the *N*-substituted ethanolamine phosphate ('1-alkenyl fragment ion') carries the charge. Further fragmentation of this fragment ion in a pseudo-MS^3 experiment using both skimmer and collision cell CID showed loss of 98 u ($=H_3PO_4$) as the main fragmentation (data not shown). This finding supports the proposed phosphomonoester structure of the '1-alkenyl fragment ion', since loss of H_3PO_4 is typical for phosphomonoesters.

As a consequence of the complementary nature of these fragments, the centre of their m/z values is located at $(m/z\ [M + H]^+ + 1)/2$, which is m/z 371.75. Hence the additional fragment ions at m/z 313.1 and 390.3 in Fig. 5 can be interpreted as indicating the presence of an isomeric structure of plasmenyl-PE(34 : 1) carrying a (16 : 0)-acyl group in position 2 and an (18 : 1)-alkenyl group in position 1 of the glycerol unit. The abundance ratios of homologous fragment ions ($[m/z\ 313]/[m/z\ 339] = 0.12$ or $[m/z\ 364]/[m/z\ 390] = 0.14$) appear to be identical within the experimental error, showing that the ion at m/z 702.5 is composed of about 87% of alkenyl-(16 : 0),acyl-(18 : 1) and 13% alkenyl-(18 : 1),acyl-(16 : 0) isomers. Hence identification of the fragment ions of protonated plasmenyl-PE shows that its complete structural characterization is possible by ESI-MS/MS.

CONCLUSIONS

Isotope-selective MS/MS is a useful tool for determination of the elemental composition of fragment ions originating from precursor ions of known elemental composition. Fragmentation of the +1 Da isotopomer of a molecular ion group provides the number of carbon atoms in the fragment ion. Fragmentation of the +2 Da isotopomer allows the estimation of the number of oxygen atoms. The presence of elements

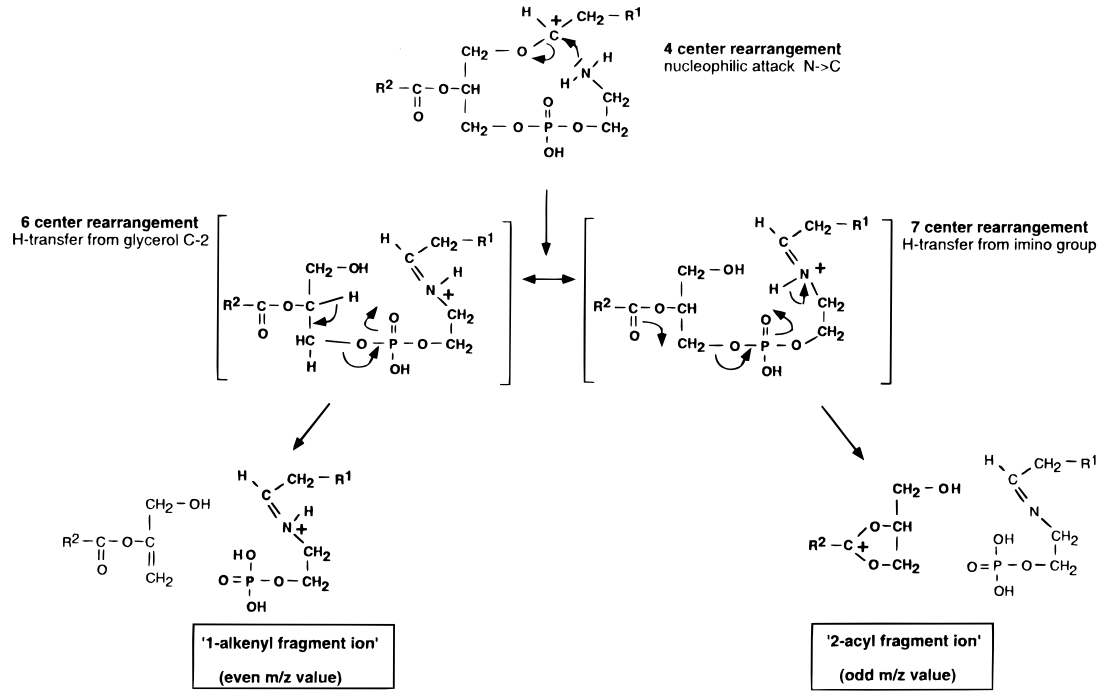

Figure 7. Proposed fragmentation pathway for protonated molecules of 1-alkenyl-2-acyl-PE. In the first step the alkenyl substituent at position 1 is transferred to the amino function forming a Schiff base. In the second step, the phosphate group is cleaved off from glycerol-C(3) accompanied by proton transfer to the phosphate group. This proton may originate either from the protonated Schiff base (the '2-acyl fragment ion' is formed) or from the glycerol structure (the '1-alkenyl fragment ion' is formed).

other than oxygen with abundant stable isotopes 2 Da heavier than the principle isotope such as sulphur (^{34}S), chlorine (^{37}C) or bromine (^{81}Br) can also be determined in this way; however, their presence interferes with the contribution of the less abundant ^{18}O isotope. The method is simple, since it can be performed on non-enriched molecules using standard triple-quadrupole instrumentation. It provides useful information on the elucidation of fragmentation pathways, in particular of those which cannot be explained by a single bond cleavage. Using the nano-ESI technique this information can be obtained with sample amounts in the low-picomole range. The method adds to established methods for fragment ion characterization such as exact mass determination and isotope labelling.

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