ELSEVIER

International Journal of Mass Spectrometry 200 (2000) 611–624

International Journal of Mass Spectrometry

Charge-remote fragmentation: an account of research on mechanisms and applications

Michael L. Gross

Department of Chemistry, Washington University, One Brookings Drive, St. Louis, MO 63130, USA

Received 11 September 2000; accepted 13 September 2000

Abstract

Charge-remote fragmentation is one class of decomposition reactions of gas-phase ions. Their discovery and applications had to await the development of soft ionization, particularly fast atom bombardment, and tandem mass spectrometry. The decompositions are particularly informative and allow functional groups to be identified and located in fatty acids, surfactants, steroids, complex lipids, and related materials. These are difficult structure assignments to make by other mass-spectrometry methods or by nuclear magnetic resonance and other spectroscopic techniques. Thus, charge-remote fragmentation fills an important need in structural chemistry. The mechanisms of charge-remote fragmentation underpin their structural utility, and they are still a matter of some debate. In this account, we discuss the discovery of charge-remote fragmentation in our laboratory. Further, we describe our efforts to understand their mechanism and to exploit their high information content in structure determinations of fatty acid and related materials. (Int J Mass Spectrom 200 (2000) 611–624) © 2000 Elsevier Science B.V.

Keywords: Charge-remote fragmentation; Tandem mass spectrometry; Fatty acids; Lipids; Fragmentation mechanisms

1. Introduction

The purpose of this article is to present an account of our efforts to understand and to develop structural applications of charge-remote fragmentation. The article is not a comprehensive review of the subject. The reader who is interested in such should consult the 1990 review by Jeanette Adams [1] or my update in 1992 [2]. Changfu Cheng and I recently submitted a review covering the period of 1992–2000 [3]. Because further review is unnecessary at this time, the present article is offered as an account of advances that principally took place in my laboratory starting in 1983.

We first observed examples of charge-remote fragmentation in a study of fatty acids in 1983 [4]. Although there were examples of this phenomenon in the literature, the reactions had not been given a title or studied systematically. Its full exploitation had to await means of introducing closed-shell ions of nonvolatile materials into the gas phase, and the recently discovered ionization method of fast atom bombardment (FAB) satisfied that need. We were fortunate to acquire for our laboratory in 1980 a Kratos three-sector mass spectrometer, the first commercial tandem-sector instrument [5]. One motivation for its design and acquisition was the study of molecules of

E-mail: mgross@wuchem.wustl.edu

PII S1387-3806(00)00372-9

biological importance. One intention was to use field desorption (FD) as a means of introducing such molecules into the gas phase and tandem mass spectrometry (MS/MS) to determine structure. Although examples were just emerging in the literature of the MS/MS of biological molecules, we had hoped that the triple-sector instrument would interface nicely to FD and would permit unambiguous selection of the precursor ion.

The world of mass spectrometry changed dramatically in 1981 with the announcement of FAB by Barber and co-workers [6]. Suddenly there was available a relatively simple and routine method for introducing biologically interesting and other nonvolatile molecules into the gas phase. It was fortunate for us that the three-sector Kratos MS50 was an ideal spectrometer to accommodate FAB and explore its opportunities with tandem mass spectrometry. The disadvantages of FAB were clear from the beginning. FAB produced little fragmentation, and the high chemical noise that is endemic to FAB often obfuscated the product-ion peaks. MS/MS induced additional fragmentation and removed most of the chemical noise. Further, Graham Cooks and his co-workers had made clear that MS/MS would be an important means of merging separation and structure determination in one mass spectrometer.

Under the aegis of a National Science Foundation (NSF) grant that established our laboratory at that time as a regional center for mass spectrometry, we installed FAB on the three-sector tandem instrument and began a systematic study of the MS/MS of biological molecules. Although the field of mass spectrometry was replete with information about radical cations that were produced by electron ionization (EI), little was known about the fragmentation of closed-shell ions with the exception of a few studies of ions produced by chemical ionization. We were intrigued by the prospects of gaining new structural information on important molecules that had been difficult to study by mass spectrometry.

I mention the NSF program to establish regional instrumentation centers because the funds it provided allowed us not only to commission the three-sector instrument but also to appoint a professional staff at the University of Nebraska to work on the development and application of mass spectrometry. In fact, Kenneth Tomer and Frank Crow [4], who were professional mass spectrometrists and assistant directors in 1983 of our mass spectrometry center, made the discovery of charge-remote fragmentation. Despite the fine contributions of many instrumentation resources, including one directed by Catherine Fenselau at Johns Hopkins University, the NSF disbanded the program in the early 1990s.

2. Early examples of charge-remote fragmentation

Although low-energy collisional activation (CA) had provided no interesting structural information on fatty-acid carboxylate anions, we were encouraged by the opportunity to use high-energy CA for this important class of molecules. As expected, FAB cleanly desorbed fatty acids as carboxylate anions ($RCOO^-$) without any detectable fragmentation. CA, however, produced a rich pattern of peaks separated by 14 u (corresponding to CH_2) [7]. The product-ion spectrum given in Fig. 1 is the first that we published on a fatty acid containing a modification (i.e. a double bond) [4]. The pattern of two intense peaks corresponds to fragment ions produced by cleavages of bonds allylic and homoallylic to the double bond. They are followed on the low-mass side by three very minor intervening peaks and then an intense peak corresponding to a product ion formed by cleavage of the allylic bond proximate to the carboxylate. These observations were exciting to us because from the beginning of my career, I was fascinated by opportunities to locate double bonds [8] and to use mass spectrometry to elucidate subtle structural features of molecules. This MS/MS method seemed to produce simple information and appeared to be general: that first report showed product-ion spectra of approximately ten other fatty acids.

As mentioned previously, there were earlier reports of fragmentation that qualifies as charge remote. Röllgen, Levsen, and co-workers [9–11] and these authors in collaboration with Boerboom, Haverkamp,

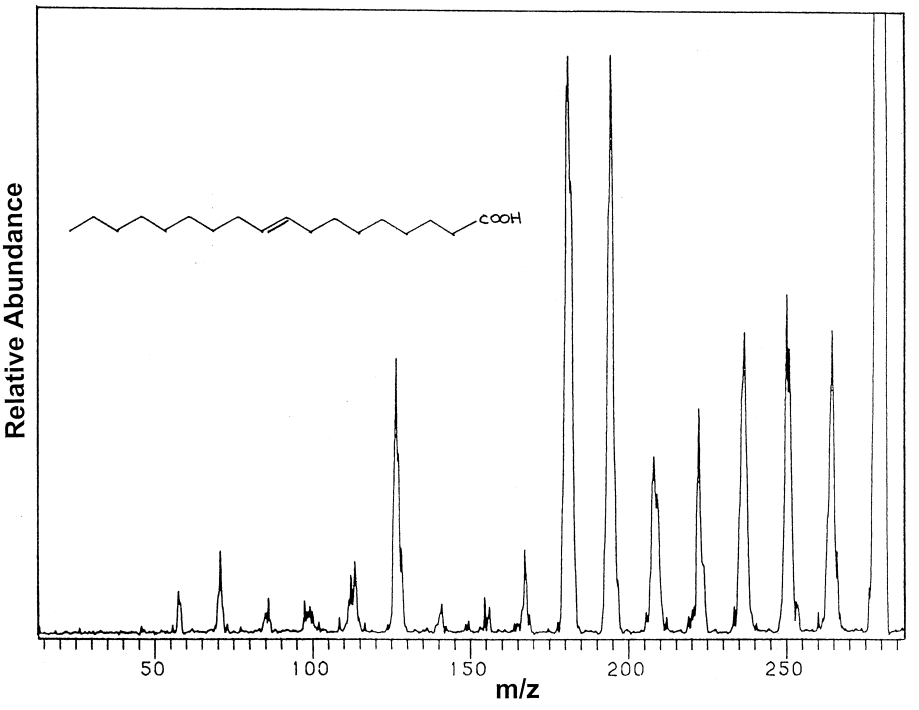


Fig. 1. High-energy, product-ion spectrum of the $[M - H]^-$ (*m/z* 281) of elaidic acid. The low mass-resolving power is typical for a three-sector, tandem mass spectrometer, which was used for this experiment. The spectrum is similar to the first spectrum, obtained in our laboratory, of an unsaturated fatty acid, revealing the opportunity to locate the functional group. The product ions at *m/z* 181 and 127 are formed by allylic cleavage/rearrangements.

and co-workers [12,13] showed some examples, as did Promé and his co-workers [14]. Bambagiotti, Traldi, and co-workers [15,16] reported clear examples of charge-remote decompositions of $[M - H]^-$ of fatty esters in 1983 and 1984. Nevertheless, these workers apparently did not recognize the generality of these reactions, nor did they pursue the topic in the extensive way that we did, as is reviewed in the following.

3. Application to modified fatty acids

Working with carboxylate anions, Nancy Jensen and Kenneth Tomer and I [7] found that we could locate double bonds in both mono and polyunsaturated fatty acids. Those with double bonds adjoining the carboxylate or those containing four or more double bonds give product-ion spectra that are difficult to interpret. In one of the earlier analytical applications, we [17] showed that reduction of the double bonds with diimide (N_2D_2) introduced two deuterium atoms in each double bond. Interpreting the mass shifts caused by incorporation of deuterium into the departing C_nH_{2n+2} neutral fragments permitted the determination of the double-bond locations in the original fatty acid. A more convenient approach, however, is to activate the fatty acid that is desorbed as $[M - H + 2Met]^+$ where Met is an alkali metal ion, as is discussed in the next section.

A striking application of charge-remote fragmentation in structural chemistry is iso and anteiso branching in fatty acids. These fatty acids are common in bacterial and marine organisms. As expected, the iso-fatty acids lose methane and the elements of propane whereas the isomeric anteiso-fatty acids undergo alkyl chain cleavages to eject methane, the elements of ethane and butane but not propane [18]. These early results convinced us that the phenomenon

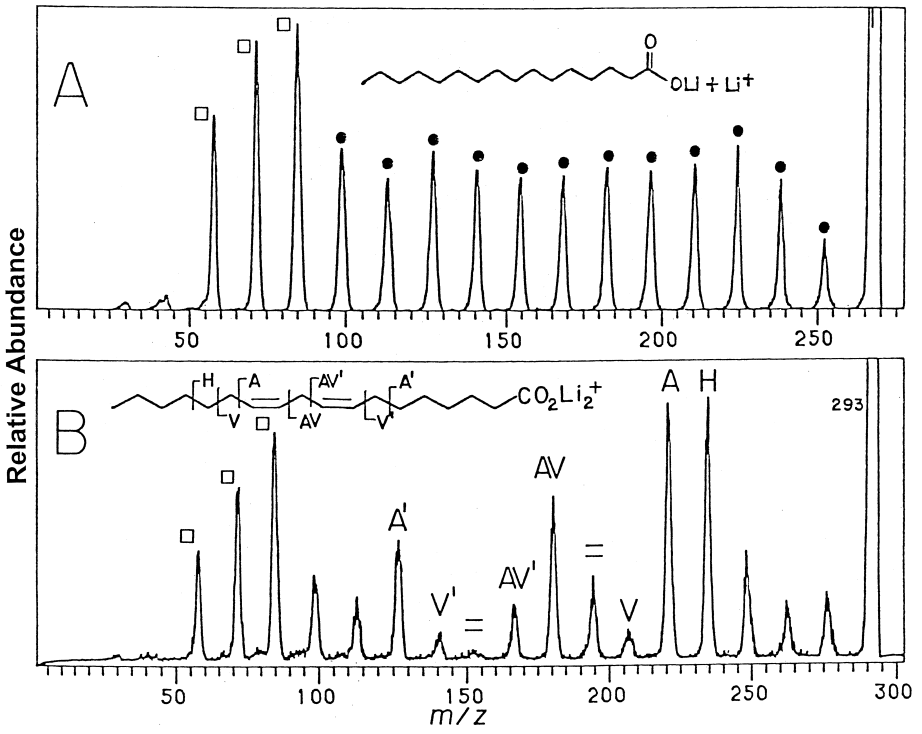


Fig. 2. (A) High-energy, product-ion spectra of the $[M - H + 2Li]^+$ of palmitic acid, showing the typical pattern for a saturated material. The peaks designated with closed circles correspond to product ions that are formed by C_nH_{2n+2} losses. (B) Product-ion spectrum of 8,11-octadecadienoic acid. H, A, and A′ represent ions formed by homoallylic and allylic-bond cleavages, respectively, whereas AV, AV′, V, and V′ represent cleavages of allylic/vinylic and vinylic bonds. The double bonds are represent by =. The spectra were taken with a three-sector tandem mass spectrometer. Reproduced from [20] with permission.

of charge-remote fragmentation had important applications in structure studies.

In addition to unmodified, unsaturated, polyunsaturated, and methyl-branched fatty acids, other modifications including the presence of other alkyl branches, hydroxy groups, cyclopropane rings, cyclopropene rings, and epoxide groups can be identified and located by interpreting the charge-remote fragmentation pattern of the unknown molecule [19].

Homoconjugated fatty acids are the most important diunsaturated fatty acids in nutrition and biochemistry. Janeen Crockett and I [20] collaborated with two well-respected lipid chemists, Dr. Ralph Holman of the Hormel Institute, and Dr. William Christie, of the University of Glasgow, who provided an invaluable series of 12 homoconjugated octadecadienoic acids (ODDA). We desorbed these isomers as $[M - H]^-$, $[M - H + 2Li]^+$, and $[M - H + Ba]^+$ and submitted them to collisional activation, allowing us to compare and contrast the relative merits of desorbing the analyte in various forms. Fig. 2 shows the striking contrast between the product-ion spectra of the saturated analog and the 8,11-ODDA and the ability to locate the homoconjugation. It is interesting that one of the double bonds appears to be cleaved, probably because it has undergone some isomerization as a consequence of the fragmentation mechanism.

One functional group that we overlooked for many years was the carbonyl group in oxyfatty acids. In 1997 in a collaborative study with Schmitz and co-workers [21], we applied charge-remote fragmentation to the structure determination of new acetylenic metabolites from a marine sponge. One of the functional groups that we were unable to locate unambiguously was the carbonyl group in this set of complex lipids. This motivated a systematic study of isomeric

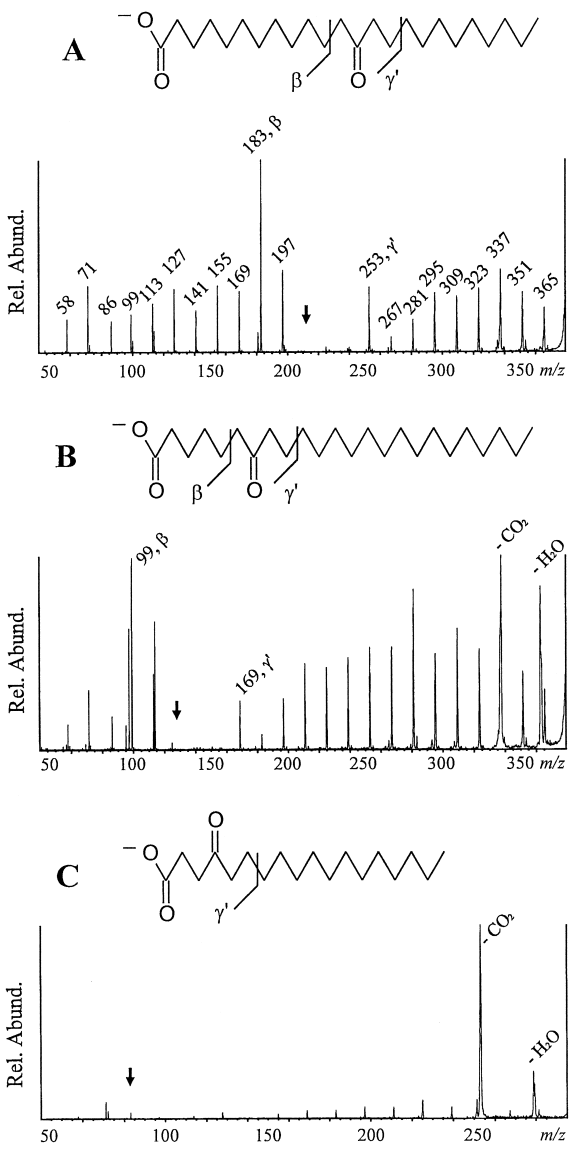


Fig. 3. High-energy, product-ion spectra of two isomers, illustrating the ease of locating the ketone group: (A) 13-oxotetracosanoate of *m/z* 381, (B) 7-oxotetracosanoate (*m/z* 381), and (C) is the product-ion spectrum of 4-oxooctadecanoate (*m/z* 297). These spectra were obtained on a four-sector tandem mass spectrometer. Taken from [22] with permission.

oxofatty acids, which was carried out by Changfu Cheng and co-workers [22]. Fig. 3 reveals that the carboxylate undergoes structurally informative charge-remote fragmentations to reveal the location of the carbonyl group. When the carbonyl is near the carboxylate [Fig. 3(C)], the nearly exclusive fragmentation is loss of CO_2, which moves the negative charge onto the alkyl chain and preempts the structurally informative charge-remote fragmentations. The product-ion spectra of metal-cationized fatty acids (see Sec. 4) do not suffer this limitation. Fig. 3 also reveals the significant advantage of using a four-sector mass spectrometer to follow charge-remote fragmentation (i.e. compare the mass resolving power exhibited by the spectra in Fig. 3 with that in Figs. 1 and 2). At this moment, it is unlikely that tandem sector mass spectrometers will experience a renaissance, and one hope is that tandem time-of-flight mass spectrometry will provide a future means of exploiting charge-remote fragmentation of fatty acids and related substances.

4. Role of the charge: alkali and alkaline-earth cationized fatty acids

The above examples suggest that the role of charge is not of primary importance in the chemical reactions that occur as charge-remote fragmentation. Two means of testing that hypothesis are to “charge” the molecule in another way and to use positive rather than negative charging. Protonation is the most commonly used ionization method, and yet protonated fatty acids do not undergo charge-remote fragmentation presumably because charge-directed loss of water and CO move the positive charge onto the alkyl chain, giving rise to a single or a set of carbocations. Carbocations are notorious for their ability to isomerize before fragmentation, and as a result, $[M + H]^+$ ions are not useful for tandem mass spectrometric structural studies of fatty acids.

Metal-cationized fatty acids, however, do produce extensive and nearly exclusive charge-remote fragmentations upon high-energy collisional activation, extending the analytical capabilities of the chemistry and providing support for the hypothesis that the processes are largely independent of charge. The product-ion spectrum of phytanic acid desorbed as $[M - H + 2Li]^+$ was obtained by Jeanette Adams and myself [23]. As a fine example of application to

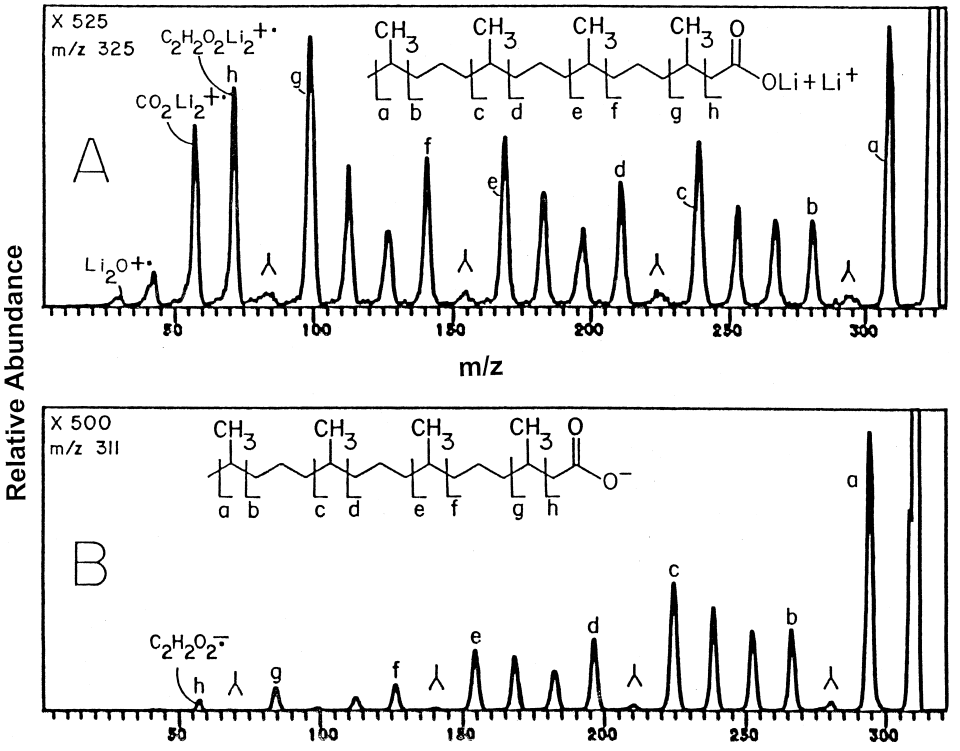


Fig. 4. High-energy, product-ion spectra of phytanic acid desorbed as (A) $[M - H + 2Li]^+$ or as (B) $[M - H]^-$. Cleavage points are indicated by letters and location of branch points by gaps in the spectrum. The spectrum, which was taken with a three-sector tandem mass spectrometer, is similar to that published in [24].

other modified fatty acids [24,25], it underscores the ability to locate all of the methyl branch sites on the molecule (Fig. 4), particularly when the metal-cationized precursor is selected. The fragmentation of the carboxylate anion is also useful, but the low-mass end of the product-ion spectrum suffers from poorer signal-to-noise ratio because the decomposition reactions are less favorable than those occurring at the end of the molecule, remote from the charge site. This phenomenon is general and forms the basis for recommending the activation, when possible, of a metal-cationized species in lieu of the carboxylate anion.

Another advantage of activating a metal-cationized species is that migration of charge onto the alkyl chain is rare. This does occur for carboxylates when the functional group is near the carboxylate anion [see Fig. 3(C)], promoting loss of carbon dioxide. Another example is the fragmentation of an α-hydroxyfatty acids, which undergo extensive loss of 46 u (presumably formic acid), a process that is more competitive than charge-remote fragmentation. Loss of 46 does not occur for the metal-cationized species.

Enrico Davoli and I [26] showed that fatty acids can also be activated as $[M - H + Met]^+$, where Met is a divalent, alkaline-earth metal ion. An advantage of using barium as the cationizing agent is that the products of charge-remote fragmentation are shifted to a higher mass, lower chemical-noise part of the product-ion spectrum. Further, the isotope pattern of barium is characteristic, and the high mass defect of barium allows ready separation of the cationized analyte from interfering ions provided, of course, that the first mass spectrometer of the tandem has moderately high resolving power. A more important aspect of this work is that the successful use of another cationizing agent supports the premise that the nature of the charge is not of high importance in these decomposition reactions.

Leesa Deterding and I [27,28] showed that fatty acids that had been derivatized with high-proton-affinity groups such as amides, pyrrolidides, and picolinyl esters fragment to give decompositions that originate remote from the charge site. This is additional evidence that any molecule with a stable charge site may undergo charge-remote processes. Further, the trends permitted us to estimate crudely, on the basis of the proton affinities of the derivitizing groups, that the internal energy required for charge-remote processes is between 1.4 and 2.9 eV. The energetic requirements are discussed in more detail in Sec. 10.

5. Surfactants and phosphonium ions

Surface-active agents, or surfactants, are widely used in commerce and are closely related to fatty acids, having a polar end and a hydrophobic tail. Often surfactants are complex mixtures of homologues, motivating the combination of desorption ionization coupled with charge-remote fragmentation for determining surfactant structures in mixture. With Philip Lyon of 3M Corporation, we identified these molecules as ideal candidates to undergo charge-remoted fragmentation. We investigated cationic [29], anionic [30], and fluoroalkane sulfonates [31]. As expected, the positive ions of amines gave patterns that are characteristic of carbocation fragmentation, owing to loss of ammonia and movement of the charge onto the alkyl chain. Quaternary amines, on the other hand, decompose, when submitted to high-energy activation, to give classic charge-remote patterns [29]. A typical anionic surfactant contains a sulfate or sulfonate polar head group, a group that would be expected to be a stable charge site. Indeed, these molecules fragment in a manner very similar to fatty-acid carboxylates [30].

Interestingly, perfluoroalkane sulfonate surfactants, when desorbed as negative ions, fragment dominately by loss of perfluoroalkyl radicals (C_nF_{2n+1}), giving possibly distonic radical anions. The losses of C_nF_{2n+2}, which would be expected to be more facile, are minor processes [31]. This propensity to lose a radical demonstrates that this loss can compete with that of the elements of an alkane and underscores the concept that loss of stable molecules is not the only one that qualifies as "charge-remote." In fatty acids, the loss of the elements of an alkane dominates, presumably because an alkane (or an alkene and H_2) are more stable than free radicals, and radical loss only occurs under circumstances where a stable free radical can be produced (e.g. simple cleavage of an allyl bond in an unsaturated fatty acid).

Some support for this argument comes from some enthalpy-of-reaction calculations of a model system. To break the middle C–C bond in *n*-butane to give two ethyl radicals requires approximately 90 kcal/mol. The fragmentation of *n*-butane to give two molecules of ethene and a molecule of hydrogen, on the other hand, has an enthalpy demand of only 57 kcal/mol at room temperature, explaining why processes similar to the latter dominate in the charge-remote fragmentation of fatty acids. On the other hand, the production of two molecules of C_2F_4 and one molecule of F_2 from perfluoro-*n*-butane requires approximately 200 kcal/mol at room temperature whereas the cleavage of the central C–C bond needs only 97 kcal/mol. Thus, a charge-remote, simple cleavage of a C–C bond can clearly compete with a rearrangement to eliminate closed-shell molecules. We will discuss this idea in more detail in Sec. 10.

Another way of locating a stable charge site in a molecule is by way of a triphenylphosphonium group. For molecules in which the fourth group bound to phosphorus is an alkyl chain, David McCrery, David Peake, and I [32] showed that the alkyltriphenylphosphonium ion will undergo classic charge-remote fragmentation upon high-energy collisional activation in a tandem mass spectrometer. We also showed that low-energy collisional activation in a Fourier transform mass spectrometer was unable to induce charge-remote decompositions of these molecules.

6. Steroids

Steroids are a diverse set of biologically important molecules for which EI has been extensively used in identification and structure proof. For those steroids

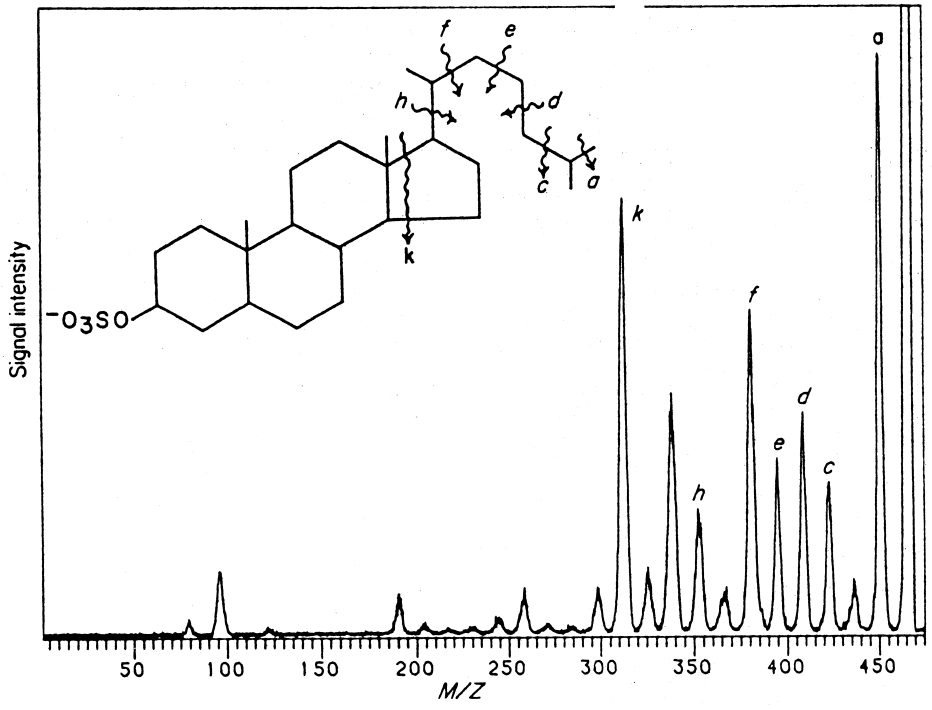


Fig. 5. Product-ion spectrum of the $[M - H]^-$ ion of cholestanol-3-sulfate, which was taken with a three-sector mass spectrometer. The fragmentation, indicated with lower case letters, occurs remote from the charge site that adjoins the a ring. Reproduced from [33] with permission.

bearing a polar or ionic group, however, FAB, electrospray, and matrix-assisted laser desorption are more appropriate ionization methods. Further, high-energy CA should cause charge-remote fragmentation to occur, providing a test for the independence of charge and reaction site. Although the nature of the charge site is of little consequence in the fragmentation of fatty acids, there is always the uncertainty that the molecule is folding, allowing the charge and reactions sites to interact. The rigid steroid ring system will prevent this folding and preclude interaction of a charge site on one of the peripheral rings with reaction sites on the other periphery.

Our first study of steroids was of bile salts with Joanne Whitney and co-workers [33]. In the article, we presented evidence that cholate and taurocholate, for example, undergo fragmentation in the A, B, and C rings even though the charge site is appended to the D ring. Even more convincing is the product-ion spectrum of cholestanol-3-sulfate (Fig. 5). The stable sulfate group is located on the A ring and yet the predominant fragmentation takes place on the alkyl appendage of the D ring and on the D ring itself.

This study led to a sequel investigation of 35 steroid conjugates (sulfates and glucuronides) and bile salts [34]. We found that stable $[M - H]^-$ ions from the steroid sulfates under high-energy collisional activation decomposed by reactions occurring remote from the charge site. The reactions are remarkably useful, even for distinction of subtly different isomers.

An important application of charge-remote fragmentation is the identification and structure proof of steroid (estrogen) adducts to nucleobases, a project carried out as a long-term collaboration with Ercole Cavalieri and his co-workers [35]. The hypothesis is that estrogens are oxidized to catechols and then to quinones, which as electrophiles, react with nucleobases on DNA, modifying them and causing mutations and ultimately breast cancer. The catechol estrogen-3,4-quinones, which are carcinogenic in

animal tests, react with guanine on DNA to cause release (depurination) of the base as an catechol-estrogen adduct. These adducts, ionized by either electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI), decompose by charge-remote processes, just as the steroids mentioned previously, to give through-ring cleavages in the B, C, and D rings even though the modification involves reaction of the nucleobase at the A ring. The ionizing proton is presumably localized on the high proton-affinity base (Gua), permitting the fragmentation reactions to occur remote to that charge site. This characteristic fragmentation chemistry is important as it allowed us to prove that the Gua adduct is formed in vitro [35].

7. Porphyrins

Porphyrins that are substituted with fatty-acid groups are another substrate that can undergo charge-remote fragmentation that characterizes the fatty-acid side chains. These materials are important as liquid crystals and in Langmuir-Blodgett films. In collaboration with M. Rosario Domingues, M. Graca S-Margues, and A.J. Ferrer-Correia of the University of Aveiro in Portugal, Olga Nemirovskiy and I [36] investigated these molecules to test the feasibility of a structural method. We also wished to see if charge-remote fragmentation occurs upon post-source decay (PSD) or collision-activated dissociation (CAD) in a MALDI time-of-flight mass spectrometer. The subject molecules were substituted tetraphenylporphyrins for which a fatty-acid moiety was attached to one or more of the phenyl groups. This opens the possibility of charge-remote fragmentation occurring along an alkyl chain attached to a porphyrin macrocycle, which can be viewed as both an “inert” support and a site for a stable charge site. We found that the PSD and CAD spectra of MALDI-produced ions are similar, showing that classic charge-remote fragmentation along an alkyl chain does occur upon PSD. This fact is in opposition with a widely held view that PSD spectra are similar to low-energy CAD spectra such as those obtained with triple-quadrupole or ion-trap instruments.

8. Complex lipids

The utility of charge-remote fragmentation in fatty-acid structure determination suggested its extension to complex lipids. In fact, FAB and tandem mass spectrometry and now ESI or MALDI mass spectrometry are productively used in structure determinations of complex lipids. The applications are numerous, but we will confine our remarks to our early work that demonstrated the analytical capabilities. In 1986, Jensen, Tomer, and I [37] published the first study of phosphatidylserines and phosphatidylcholines. Phosphatidylcholines, in the negative-ion mode, do not give an $[M - H]^-$, but rather desorb, accompanied by various positive group transfer reactions, to give three characteristic high-mass precursor ions: $[M - CH_3]^-$ (presumably a methyl transfer to matrix), $[M - HN(CH_3)_3]^-$, and $[M - HN(CH_3)_3 - C_2H_2]^-$ (see Fig. 6 for structures). When collisionally activated, these ions release the constituent fatty-acid anions, which are also formed to a small extent upon FAB desorption. The fatty-acid carboxylates can be activated in a subsequent experiment to reveal their structures. For example, in Fig. 6, we see the product-ion spectrum of these three ions produced upon FAB. The fragments at *m/z* 283 and 303 are the two constituent fatty-acid carboxylates. Although the fatty acid in position 2 is usually liberated as the more abundant carboxylate, as illustrated by Fig. 6(C), this is not always the case. Huang et al. [38], in a study of many more phospholipids than we used in the original work, showed that the $[M - HN(CH_3)_3 - C_2H_2]^-$ always expels more favorably as ketene, the fatty acid in the two position, giving a trustworthy source of information on the location of the two fatty acids. Another MS/MS experiment is needed to probe the structures of the two constituent fatty acids, either by choosing the carboxylate produced by FAB or by using a MS^3 experiment, which is difficult on tandem-sector instruments.

The significance of the analytical advance made possible by this work is that structure determination of

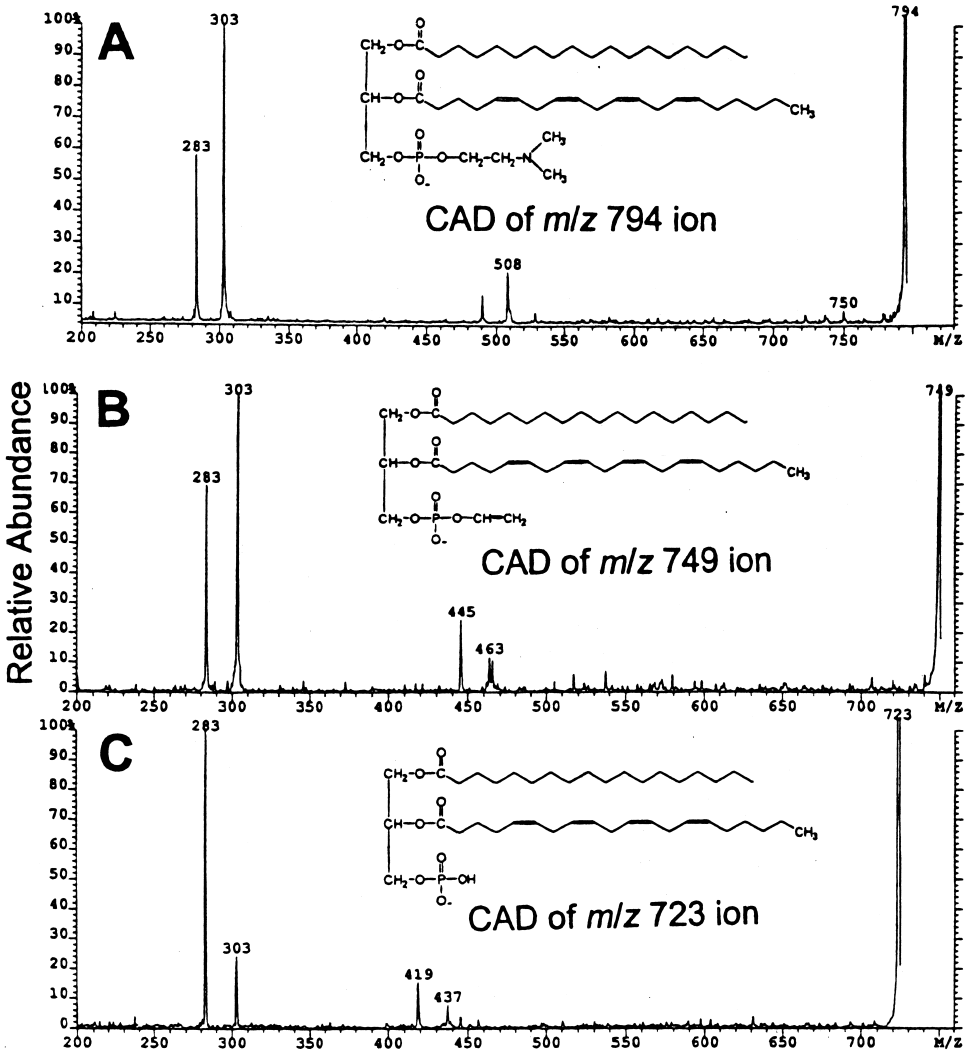


Fig. 6. Product-ion spectra of the three precursor ions formed upon FAB of the phosphatidylcholine (C18:0/C20:4). Note the two abundant product ions of *m/z* 283 and 303, which are the two constituent carboxylates and the less abundant, higher *m/z* ions that are formed by elimination of a fatty acid as a ketene.

complex lipids can be accomplished by an entirely instrumental method either in combination with sample introduction by liquid chromatography or by infusion of simple mixtures of the complex lipids. This advantage also applies to other complex lipids such as those with inositol, glycerol, ethanolamine, and others as the polar group [39].

An unusual complex lipid that we studied in 1986 contained the amino acid ornithine as the polar head group, and one of the constituent fatty acids had a cyclopropane ring [40]. We mention this here because we were able to use charge-remote fragmentation to confirm the structure of the principal component and determine that of a minor homolog. Fragmentation of the fatty-acid carboxylate, which was released upon FAB desorption, produces a facile loss of 42 u, consistent with its assignment as a α-hydroxy acid. Nevertheless, charge-remote decompositions produced sufficiently abundant product ions to locate the cyclopropyl ring in the major component. The minor

component was another ornithine-containing lipid whose molecular mass was 14 u smaller than that of the major component. We collisionally activated that component and determined that the mass difference was in the fatty acid of the major component that contained the cyclopropyl ring. Specifically, we could use the product-ion spectrum of the minor component to show that the only difference in the molecule was that the cyclopropyl ring had been replaced with a double bond.

Perhaps the most common lipid materials are triglycerides. Recently, Changfu Cheng and I, in collaboration with Ernst Pittenauer in Vienna [41], developed a MS/MS method that elucidates the complete structure of triacyglycerols. The method works for $[M + NH_4]^+$ and $[M + Met]^+$, where Met is an alkali metal ion. These ions can be introduced into the mass spectrometer by either ESI or FAB, although the product-ion spectra of FAB-produced ions are more informative. Nevertheless, interpretation of the product-ion spectra gives information of the number of carbon atoms, the degrees of unsaturation, the location of double bonds, and the positions of the various fatty acids on the glycerol backbone.

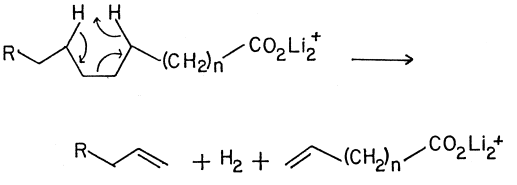
9. Other applications

As mentioned in Sec. 1, this article is not intended as a comprehensive review. Other reviews [1–3] are available, and the interested reader is directed to them for a comprehensive treatment of the subject. Nevertheless, it is appropriate to mention briefly the broad range of chemical substances that undergo structurally informative charge-remote fragmentations. Prostaglandins and leukotrienes originate from fatty acids, and not surprisingly, charge-remote fragmentation is an important tool in their structure determination [42]. Peptides are a class of biomolecules that have captured the imagination of many mass spectrometrists. The presence of high proton-affinity amino acids or the introduction of proper modification allows charge-remote fragmentation to occur for this important class of biomolecules. There are also examples in oligonucleotide, carbohydrate, and glycoside mass spectrometry. The compounds most expected to undergo charge-remote fragmentation, however, have features of surfactants and fatty acids. Thus, it is not surprising that vitamins, fatty alcohols, glycolipids, and ceramides are examples where charge-remote fragmentation can play a role in compound identification and structure determination.

Charge-remote fragmentation is not as common as charge-driven or radical-driven processes. Nevertheless, when the mass spectrometrist is faced with a structure proof of a class of compounds that undergo charge-remote fragmentation or one that can be derivatized to provide a stable charge site, he/she is well advised to take advantage of this informative class of gas-phase decompositions.

10. Mechanism and energetics

Considerations of mechanism and energetics are most usefully defined when one realizes that there are many classes of charge-remote processes. The process that was first identified is the cleavage/rearrangement along alkyl chains such as in fatty acids. Nancy Jensen, Kenneth Tomer, and I first proposed and provided evidence for a 1,4-elimination mechanism (Scheme 1) in 1985 [7]. Some of the key observations



Scheme 1.

abstracted from much of the phenomena and applications described above provide a foundation for this proposal. First, the nature of the charge is not important. The charge site can be positive or negative. It can be a carboxylate, a sulfate, sulfonate, a metal-cationized fatty acid or alcohol, an ammonium or phosphonium ion, or even the macrocyclic ring of a porphyrin. This wide variety of charge carriers and charge states

shows that charge is not important in the charge-remote reactions.

This conclusion is underscored by observations that steroids charged at end one of the molecule undergo decompositions at a remote site where interaction with the charge site is not possible, as discussed previously. Changfu Cheng and I [43] recently reported that an oxofatty acid linked to a steroid in such a way that charge and reaction sites cannot interact undergoes charge-remote fragmentations that are nearly identical to those of the oxofatty acid itself.

The other evidence is that the fragments formed by reactions along an alkyl chain are a set of homologous C_nH_{2n+2}. If these were alkanes, one would expect the two sets of ionic products for cleavages along an alkyl chain to depend on the direction of the hydrogen transfer that accompanies the rearrangement. One set would be saturated or contain alkyl groups; the other would be unsaturated and contain alkenyl groups. Only one class of ionic product is formed; that is one with an unsaturation (i.e. an alkenyl group). This observation is consistent with a 1,4-H_2 elimination (and with other mechanisms). A third piece of evidence that is consistent with the 1,4-H_2 elimination comes from the results of deuterium labeling, but these results are not proof that the process is a 1,4-H_2 elimination.

A feature of the 1,4-H_2 elimination is that its energy requirement should be low because the mechanism incorporates both bond breaking and bond making. To explore this possibility, Jeanette Adams and I [44] designed a set of experiments to measure the energy requirement for the charge-remote cleavages along an alkyl chain of a fatty alcohol. We desorbed the fatty alcohols as $[M + Met]^+$, where Met was Li, Na, K, Rb, or Cs because we could approximate the affinities of the alcohol for these alkali metal ions. We found, for example, that $[M + Cs]^+$ fragmented exclusively by release of Cs^+ whereas $[M + Na]^+$ and $[M + K]^+$ gave hints that charge-remote decompositions compete with the release of the metal ion itself. These observations coupled with the known metal ion affinities of simple alcohols permitted an estimate of the energy requirement for charge-remote cleavage of a C–C bond to be between 1.3 and 1.9 eV.

We feel that this estimate, which is approximately one half a C–C bond energy, is consistent with the 1,4-elimination reaction. One is not surprised that the bond would be approximately half broken in a transition structure; the 1,4-elimination involves bond making in concert with bond breaking. Further, the involvement of six electrons makes the transition structure aromatic-like, stabilizing it and promoting the reaction at energies that would be less than those require to cleave a C–C or C–H bond to give an intermediate free radical.

Strong support for the 1,4-H_2 elimination could be achieved from the identification of the neutral products formed in the charge-remote fragmentation along an alkyl chain. Chrys Wesdemiotis and co-worker [45] were able to characterize the neutral products by neutral fragment-reionization mass spectrometry. They found that the neutral products are alkenes and not alkyl radicals or alkanes, a finding that is consistent with the proposed 1,4-elimination process.

If the reactions are charge-remote (have little or no dependence on charge), then they should be analogous to thermal reactions. Research by Jeanette Adams and I [46] showed that the well-known thermal reactions, O-hydro-C-allyl and hydro-hydroxy eliminations occur in fatty-acid templates designed to carry the appropriate functional groups to permit the reactions. Thermal retro-ene and 1,4-conjugate eliminations may be responsible for the cleavage of allylic bonds in unsaturated fatty acids [47]. These proposals, however, have an undesired outcome that a different mechanism applies to the reaction occurring at a functional group proximate to the charge site compared to that occurring remote to the charge site. Even the 1,4-H_2 elimination to give C–C bond cleavage along a saturated fatty-acid chain has an analogy in the 650 °C pyrolysis of fatty-acid esters. Like the charge-remote process in a tandem mass spectrometer, the thermal reaction gives a series of alkenes and unsaturated fatty-acid esters [48].

Nevertheless, efforts to prove mechanism in chemistry are never complete, and a satisfactory mechanism must account for all the experimental data. The

first challenge to the 1,4-elimination mechanism was by Wysocki and Ross [49], who proposed a two-step process in which the first step was a homolytic cleavage of a C–C bond. Magda Claeys and her co-workers [50–55] reported the second challenge in a series of recent articles. They proposed a new mechanism that involves a C–H bond cleavage as the dominant step. Both C–H and C–C cleavages place a radical site on the alkyl chain as a distonic ion, explaining the production of simple-cleavage products that are occasionally seen. The radical site then triggers cleavages to produce the even-electron ions that are most common in charge-remote fragmentation. The fact that both odd and even-electron ions are formed may be due to interactions between the reaction center and the charge site, particularly when the charge is provided by an alkali-metal ion. Of course, this interaction is at odds with the charge-remote concept. They also observed for fatty-acid esters cationized with Na^+ or Li^+ a facile loss of H_2 that occurs randomly along the fatty-acid chain, producing a mixture of monounsaturated fatty ester ions. The easy loss of H_2 may occur by hydrogen radical removal, and if so, supports the argument that, to some extent, that charge-remote reactions start with C–H cleavage. Claeys and co-workers also find isotope effects that are consistent with rate-determining C–H cleavage. The 1,4-H_2 elimination, however, also involves C–H bond breaking in its transition structure and should show a kinetic isotope effect.

11. Conclusion

Charge-remote fragmentation is not only a valuable approach to structure determination of fatty acid, steroid, and related molecules but also an important intellectual concept for gas-phase ion chemistry. It serves as a major classification of those chemical reactions that occur for gas-phase ions. The mechanism and energy requirements for the “classic” reaction that occurs along an alkyl chain are still the subject of investigation although we prefer to believe that most occur via a 1,4-H_2 elimination. The work of Claeys and co-workers strives for a single mechanism that explains all products; this reaction begins with a homolytic bond cleavage to produce a radical site on an alkyl chain. We acknowledge that homolytic cleavages occur. Indeed, they become competitive as the internal-energy states change in the precursor ions [56] and even become dominant in fluoroalkane sulfonates (discussed earlier). Nevertheless, we prefer to hold with the proposal that these reactions occur in parallel with the 1,4-H_2 elimination rather than in series with it.

There undoubtedly are many charge-remote processes including the C–C bond cleavage/rearrangement, perhaps rivaling the number of thermal processes in chemistry. Thus, there should remain numerous opportunities to develop structural approaches and to explore mechanisms for fragmentation centered on various functional groups. An example is our recent study of the mechanism for charge-remote fragmentation centered at ketone groups in fatty-acid chains [43].

Acknowledgement

The National Centers for Research Resources of the NIH supported the preparation of this account (Grant no. 2P41RR00954).

References

[1] J. Adams, Mass Spectrom. Rev. 9 (1990) 141.
[2] M.L. Gross, Int. J. Mass Spectrom. Ion Processes 118–119 (1992) 137.
[3] C. Cheng, M.L. Gross, Mass Spectrom. Rev., in press.
[4] K.B. Tomer, F.W. Crow, M.L. Gross, J. Am. Chem. Soc. 105 (1983) 5487.
[5] M.L. Gross, E.K. Chess, P.A. Lyon, F.W. Crow, S. Evans, H. Tudge, Int. J. Mass Spectrom. Ion Phys. 42 (1982) 243.
[6] M. Barber, R.S. Bordoli, R.D. Sedwick, A.N. Tyler, J. Chem. Soc., Chem. Commun. (1981) 325.
[7] N.J. Jensen, K.B. Tomer, M.L. Gross, J. Am. Chem. Soc. 107 (1985) 1863.
[8] M.L. Gross, P.-H. Lin, S.J. Franklin, Anal. Chem. 44 (1972) 974.
[9] F.W.K. Röllgen, F. Borchers, U. Giessmann, K. Levsen, Org. Mass Spectrom. 12 (1977) 541.
[10] H.H. Gierlich, F.W.K. Röllgen, F. Borchers, K. Levsen, Org. Mass Spectrom. 12 (1977) 387.

[11] R. Weber, F. Borchers, K. Levsen, F.W.K. Röllgen, Z. Naturforsch. 33a (1978) 540.
[12] R. Weber, K. Levsen, G.J. Louter, A.J.H. Boerboom, J. Haverkamp, Anal. Chem. 54 (1982) 1458.
[13] R. Weber, K. Levsen, A.J.H. Boerboom, J. Haverkamp, Int. J. Mass Spectrom. Ion Phys. 46 (1983) 305.
[14] G. Puzo, B. Maxime, J.C. Promé, Int. J. Mass Spectrom. Ion Phys. 48 (1983) 169.
[15] A.M. Bambagiotti, S.A. Coran, V. Giannellini, F.F. Vincieri, S. Daolio, P. Traldi, Org. Mass Spectrom. 19 (1984) 577.
[16] A.M. Bambagiotti, S.A. Coran, V. Giannellini, F.F. Vincieri, S. Daolio, P. Traldi, Org. Mass Spectrom. 18 (1983) 133.
[17] N.J. Jensen, K.B. Tomer, M.L. Gross, Anal. Chem. 57 (1985) 2018.
[18] N.J. Jensen, M.L. Gross, Lipids 21 (1986) 362.
[19] K.B. Tomer, N.J. Jensen, M.L. Gross, Anal. Chem. 58 (1986) 2429.
[20] J.S. Crockett, M.L. Gross, W.W. Christie, R.T. Holman, J. Am. Soc. Mass Spectrom. 1 (1990) 183.
[21] X. Fu, S.A. Abbas, F.J. Schmitz, I. Vidavsky, M.L. Gross, M. Laney, R.C. Schatzman, R.D. Cabuslay, Tetrahedron 53 (1997) 799.
[22] C. Cheng, D. Giblin, M.L. Gross, J. Am. Soc. Mass Spectrom. 9 (1998) 216.
[23] J. Adams, M.L. Gross, Anal. Chem. 59 (1987) 1576.
[24] J. Adams, M.L. Gross, Org. Mass Spectrom. 23 (1988) 307.
[25] J. Adams, L.J. Deterding, M.L. Gross, Spectroscopy (Ottawa) 5 (1988) 199.
[26] E. Davoli, M.L. Gross, J. Am. Soc. Mass Spectrom. 1 (1990) 320.
[27] L.J. Deterding, M.L. Gross, Org. Mass Spectrom. 23 (1988) 169.
[28] L.J. Deterding, M.L. Gross, Anal. Chim. Acta 200 (1987) 431.
[29] P.A. Lyon, F.W. Crow, K.B. Tomer, M.L. Gross, Anal. Chem. 56 (1984) 2278.
[30] P.A. Lyon, W.L. Stebbings, F.W. Crow, K.B. Tomer, D.L. Lippstreu, M.L. Gross, Anal. Chem. 56 (1984) 8.
[31] P.A. Lyon, K.B. Tomer, M.L. Gross, Anal. Chem. 57 (1985) 2984.
[32] D.A. McCrery, D.A. Peake, M.L. Gross, Anal. Chem. 57 (1985) 1181.
[33] K.B. Tomer, N.J. Jensen, M.L. Gross, J. Whitney, Biomed. Environ. Mass Spectrom. 13 (1986) 265.
[34] K.B. Tomer, M.L. Gross, Biomed. Environ. Mass Spectrom. 15 (1988) 89.
[35] E.L. Cavalieri, D.E. Stack, P.D. Devanesan, R. Todorovic, I. Dwivedy, S. Higginbotham, S.L. Johansson, K.D. Patil, M.L. Gross, J.K. Gooden, R. Ramanathan, R.L. Cerney, E.G. Rogan, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 10937.
[36] M. Rosario, M. Domingues, O.V. Nemirovskiy, M. Graca, S. Marques, C.A.M. Vale, M. Graca Neves, J.A.S. Cavaleiro, A.J. Ferrer-Correia, M.L. Gross, J. Am. Soc. Mass Spectrom.
[37] N.J. Jensen, K.B. Tomer, M.L. Gross, Lipids 21 (1986) 580.
[38] Z.H. Huang, D.A. Gage, C.C. Sweeley, J. Am. Soc. Mass Spectrom. 3 (1992) 71.
[39] N.J. Jensen, K.B. Tomer, M.L. Gross, Lipids 22 (1987) 480.
[40] R.L. Cerny, K.B. Tomer, M.L. Gross, Org. Mass Spectrom. 21 (1986) 655.
[41] C. Cheng, M.L. Gross, E. Pittenauer, Anal. Chem. 70 (1998) 4417.
[42] J.A. Zirrolli, E. Davoli, L. Bettazzoli, M. Gross, R.C. Murphy, J. Am. Soc. Mass Spectrom. 1 (1990) 325.
[43] C. Cheng, M.L. Gross, J. Am. Soc. Mass Spectrom. 9 (1998) 620.
[44] J. Adams, M.L. Gross, J. Am. Chem. Soc. 108 (1986) 6915.
[45] M.M. Cordero, C. Wesdemiotis, Anal. Chem. 66 (1994) 861.
[46] J. Adams, M.L. Gross, J. Am. Chem. Soc. 111 (1989) 435.
[47] M.J. Contado, J. Adams, N.J. Jensen, M.L. Gross, J. Am. Soc. Mass Spectrom. 2 (1991) 180.
[48] K.K. Sun, H.W. Hayes, R.T. Holman, Org. Mass Spectrom. 3 (1970) 1035.
[49] V.H. Wysocki, M.M. Ross, Int. J. Mass Spectrom. Ion Processes 104 (1991) 179.
[50] M. Claeys, H. Van den Heuvel, Biol. Mass Spectrom. 23 (1994) 20.
[51] M. Claeys, L. Nizigiyimana, H. Van den Heuvel, P.J. Derrick, Rapid Commun. Mass Spectrom. 10 (1996) 770.
[52] M. Claeys, H. Van den Heuvel, S. Chen, P.J. Derrick, F.A. Mellon, K.R. Price, J. Am. Soc. Mass Spectrom. 7 (1996) 173.
[53] L. Nizigiyimana, H. Van Den Heuvel, M. Claeys, J. Mass Spectrom. 32 (1997) 277.
[54] M. Claeys, L. Nizigiyimana, H. Van Den Heuvel, I. Vedernikova, A. Haemers, J. Mass Spectrom. 33 (1998) 631.
[55] L. Huysmans, L. Nizigiyimana, H. Van den Heuvel, M. Claeys, Int. J. Mass Spectrom. 188 (1999) 39.
[56] C. Cheng, E. Pittenauer, M.L. Gross, J. Am. Soc. Mass Spectrom. 9 (1998) 840.