7

Handbook of Lipid Research

Mass Spectrometry of Lipids

Robert C. Murphy

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Volume 1	*Fatty Acids and Glycerides* *Edited by Arnis Kuksis*
Volume 2	*The Fat-Soluble Vitamins* *Edited by Hector F. DeLuca*
Volume 3	*Sphingolipid Biochemistry* *Julian N. Kanfer and Sen-itiroh Hakomori*
Volume 4	*The Physical Chemistry of Lipids: From Alkanes to Phospholipids* *Donald M. Small*
Volume 5	*The Phospholipases* *Moseley Waite*
Volume 6	*Glycolipids, Phosphoglycolipids, and Sulfoglycolipids* *Edited by Morris Kates*
Volume 7	*Mass Spectrometry of Lipids* *Robert C. Murphy*

To my family

Carol
Beth
Geoff

Preface

Mass spectrometry in its many forms has played an important role in lipid biochemistry, serving as a tool to elucidate structures of lipids, as an essential component of quantitative analysis, and as a means to follow complex enzymatic reactions by providing information concerning transition states. This instrument-based technique is different in many respects from all other spectroscopic methods. Mass spectrometry is based on the chemistry, albeit gaseous ion chemistry, of the lipid substance. Other techniques such as nuclear magnetic resonance, infrared, ultraviolet, and x-ray spectroscopy are based on physical properties of lipids. These methods study the interaction of electromagnetic radiation with molecules through alterations in rotation, vibration, and excitation as well as scattering events. Mass spectrometry engages physics through separation of gaseous ions depending upon velocity and momentum, ultimately resulting in a measurement of mass-to-charge ratio (m/z). Physical processes are also used to form gaseous ions through electronic excitation of neutral gases and, more recently, desorption of gaseous ions from solutions.

Most, if not all, of the wealth of structural information provided by this technique is contained in subsequent decomposition (fragmentations) and rearrangements that occur as the result of intramolecular reaction of gaseous ions. These reactions are invariably unimolecular because of the very low pressures (high vacuum) employed in the mass spectrometer. The instrument allows us to observe chemical reactions of ions when the mass of the product differs from the mass of the reagent or precursor ion. The yield from reaction pathway is related to the abundance of a particular ion, which leads to the general method of graphic presentation of mass spectral data, i.e., abundance of an ion related to the mass-to-charge ratio. Finally, mass spectrometry is a quintessential technique in terms of sensitivity. It is perhaps this feature that has been singularly responsible for the application of mass spectrometry in biology and lipid biochemistry.

This book was written to provide a background in the general physical concepts of mass spectrometry (Chapters 1–3). Subsequent chapters discuss the unique ion chemistry of specific lipids, from the simplest fatty acid to complex glycosphingolipids. An understanding of the ion chemistry is essential for any student of mass spectrometry in order to apply this technique to a unique question in lipid biochemistry. Therefore, a great deal of effort has been put into presenting complete mass spectral data as well as discussing the numerous and almost always competing unimolecular reactions that support the observed frag-

mentations and rearrangements in the complex mass spectrum. When possible, reference to the original work is given, which employs different strategies to substantiate these reactions. For some situations, I have hypothesized reaction mechanism with the anticipation that these suggestions will stimulate future investigators to disprove my hypothesis and formulate a better understanding of the ion chemistry of lipids.

Any book concerning the mass spectrometery of lipids is inherently incomplete because of the number of advances and applications that continue to be reported in the primary literature. The arbitrary choice of lipids discussed in this monograph reflects the interest, experience, and training of the author. Missing from this book is a discussion of the mass spectrometry of steroids, vitamins, pheromones, and other complex lipids such as lipopolysaccharides. Inclusion of these topics would have made this book substantially larger and my task more difficult. The focus on mass spectrometry of fatty acids and fatty acyl–containing lipids encompasses a large number of lipids about which detailed studies of the relevant ion chemistry are available. It is hoped that the methods discussed and ancillary mass spectrometry strategies presented will provide useful insight for investigators of other important lipid compounds.

The discipline of mass spectrometry has exploded within the past decade, as have other areas of biomedical research. Mass spectrometry has expanded from a technique that could handle only gaseous compounds or compounds made into gases following chemical derivatization and heating into a technique suitable for the analysis of complex, nonvolatile lipids. The molecular weight of a lipid suitable for analysis is no longer limited to 500–1000 daltons; compounds with molecular weights up to 5000 daltons can be analyzed with fast atom bombardment ionization and modern sector instruments. Above 5000 and up to 200,000 daltons, electrospray ionization and matrix-assisted laser desorption techniques have been successful. Covalent modification of proteins and nucleic acids by lipid moieties is now amenable to direct mass spectrometric analysis. Development of instrumentation has also kept pace. Tandem mass spectrometry (MS/MS) with triple quadrupole or four-sector magnetic instruments extends our understanding of events taking place in lipid mass spectrometry. These new areas are discussed in some detail, with specific examples of more complex lipids, because they are the present and future of mass spectrometry.

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Acknowledgments

When approached by Fred Snyder, the editor of this series, to write a book on the mass spectrometry of lipids, I had some hesitation. Although I had always wanted to write a book on this particular topic, I knew that it would be a demanding task. I want to thank Dr. Snyder for persuading me to take on this project, and for his encouragement and his comments concerning the manuscript. Financial support from the Heart, Lung, and Blood Institute of the National Institutes of Health (HL25785 and HL34303) has been critical for my continued work in the mass spectrometry of lipids, especially eicosanoids. Their interest and support for my research efforts is most gratefully acknowledged.

This book would not have been possible without the contributions of many individuals. These include those who have made detailed studies of the ion chemistry of lipids as well as those who have developed newer analytical techniques of mass spectrometry, including tandem mass spectrometry and methods to analyze nonvolatile molecules. Professor Michael Gross (University of Nebraska) provided previously unpublished data for this book using a four-sector mass spectrometer to obtain data concerning remote site fragmentation of fatty acids. His contribution is gratefully acknowledged. Some mass spectra presented were adapted from published histograms that were computer scanned directly from the literature and then redrawn and annotated using computer drawing programs. This work was carried out by Geoffrey R. Murphy, and his professional skill and diligence in making these important figures is greatly acknowledged. Leigh Landskroner (Illustration and Photography, National Jewish Center for Immunology and Respiratory Medicine) prepared the original drawings for Figures 1-1, 1-5, 1-10, and 2-1 which very creatively captured some complex instrumentation used in mass spectrometry.

Many mass spectra in this book, particularly fatty acid derivatives and most eicosanoids, were recorded by John Simpson using a quadrupole GC/MS instrument. His careful attention to the quality of these data is especially appreciated. Kathleen Kayganich ran virtually all of the phospholipid mass spectra and most of the tandem quadrupole mass spectrometric experiments depicted in this book. Her assistance is gratefully acknowledged. Individuals from my laboratory throughout the past 20 years have provided mass spectral data through their thesis investigations. These include Timothy Harper, Rod Mathews, Peter Haroldsen, Danny Stene, and Michael Shirley.

I would like to particularly thank Dr. Joseph Zirrolli, who read the entire

manuscript, checked each mass assignment, and thought carefully about each reaction mechanism. His hard work made this book possible. Also, individuals in my laboratory went over each chapter of the manuscript in a series of meetings that revealed subtle errors, overstatements, and many areas needing further clarification. I hope they learned as much as I. These individuals include Keith L. Clay, Elizabeth Hill, Bernard Fruteau-de-Laclos, Chris Johnson, Kathleen Kayganich, Denise MacMillan, John Simpson, and Patricia Wheelan. I greatly appreciate the efforts of Dr. Catherine Costello (MIT) for her detailed review of Chapter 8, on sphingolipids. Her experience in the mass spectrometry of these lipids was especially useful.

Finally, this book would not have been completed without the professional abilities, skill, and patience of Deborah Beckworth. She prepared the entire manuscript from dictation, corrected spellings, prepared reference lists, and cheerfully worked through all of the corrections even when presented with the seventh and eighth revisions. It is not enough to express thanks; I do hope that in some way the challenge of preparing this book was fulfilling to her.

Abbreviations

Abbreviations of mass spectrometry nomenclature (see IUPAC recommendations, 1991, *Pure Appl. Chem.* 63:1541) and other abbreviations used in this volume are as follows:

B/E	A linked scan performed on a high-resolution instrument where the magnetic field-strength (B) and the electric field-strength (E) are simultaneously scanned, holding the accelerating voltage (V) constant. This experiment yields a product ion scan.
B^2/E	A linked scan experiment as above except B^2 and E are simultaneously scanned. This experiment yields a precursor ion scan.
CI	Chemical ionization. The formation of ionized species when a neutral gaseous molecule interacts with an ion species to transfer an electron, a proton, or other charged species between the reactants.
CID	Collision-induced dissociation. A process whereby a projectile ion is induced to decompose through interaction with a target neutral gas through conversion of a portion of the translational energy of the ion to internal energy of the ion. (The abbreviation CAD is also used for collision-activated decomposition.)
DCI	Desorption chemical ionization.
ECI	Electron capture ionization. Formation of a negatively charged ion species when a gaseous molecule interacts with a thermal electron that is then retained in an empty molecular orbital of the newly formed ion. The mechanism of electron capture is generally considered to be resonance capture of thermal electrons e_t^-.
eV	Electron volt; 96.5 kJ/mol; 23.1 kcal/mol.
EI	Electron ionization.
FAB	Fast atom bombardment.
GC-MS	Tandem gas chromatography–mass spectrometry.
GPA	1,2-diradyl-*sn*-glycero-3-phosphatidic acid.
GPC	1,2-diradyl-*sn*-glycero-3-phosphocholine.
GPE	1,2-diradyl-*sn*-glycero-3-phosphoethanolamine.
GPG	1,2-diradyl-*sn*-glycero-3-phosphoglycerol.
GPI	1,2-diradyl-*sn*-glycero-3-phosphoinositol.
GPS	1,2-diradyl-*sn*-glycero-3-phosphoserine.
HETE	Monohydroxyeicosatetraenoic acid.

HPLC	High-pressure liquid chromatography or high-pressure liquid chromatograph.
HPLC-MS	Tandem high-pressure liquid chromatography–mass spectrometry.
LC-MS	Liquid chromatography–mass spectrometry, synonymous with HPLC-MS.
LCB	Long-chain base.
LT	Leukotriene.
$M^{\ddagger}$	Molecular ion. The ionized intact molecule containing a radical site and a positive charge site with only the isotopes of greatest natural abundance.
$[M + H]^+$	The ionized molecule containing an additional covalently attached proton, retaining a positive charge and only the isotopes of greatest natural abundance.
$[M - H]^-$	The ionized molecule having lost one hydrogen atom, retaining a negative charge and only the isotopes of greatest natural abundance.
MS/MS	Tandem mass analyzers used for the separation and identification of ions in a single instrument typically following collision-induced dissociation of ions between the mass analyzers.
MW	Molecular weight.
m/z	Mass-to-charge ratio. Used to denote the dimensionless value obtained by dividing the mass number of an ion by its charge number.
n-electrons	Nonbinding electrons.
PFB	Pentafluorobenzyl [$C_6F_5CH_2$] moiety. Used as a derivative with high electron capture cross section.
PG	Prostaglandin.
sn-1, *sn*-2	Stereospecifically numbered. The nomenclature system describing the substituent at the C-1 or C-2 position in glycerol esters.
TLC	Thin-layer chromatography.
TMS	Trimethylsilyl [$(CH_3)_3Si$-].
u	Atomic mass unit also designated by the term dalton (Da) which is $\frac{1}{12}$ of the mass of a single (neutral) ^{12}C atom.

Contents

Chapter 3

Sample Introduction

Chapter 4

Fatty Acids

Chapter 6

Triacylglycerols, Diacylglycerols, and Monoacylglycerols

Chapter 7

Glycerophospholipids

Chapter 8

Sphingolipids