
A GUIDE TO

PHOSPHOLIPID
CHEMISTRY

DONALD J. HANAHAN

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*To Lillian,
a constant and always
supportive companion*

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PREFACE

Several years ago, I authored a book, together with Irving Zabin and Frank Gurd, entitled simply *Lipide Chemistry* (Wiley & Sons, New York, 1960). The concern expressed at that time was that studies on lipids centered on establishing the etiology and cure for atherosclerosis and other lipid-related diseases. It was an understated feeling at that time that many investigators used "seriously outmoded or incompletely tested methods or concepts in their enthusiastic search to a solution to these perplexing problems." In essence, there was a concern that many students (and senior investigators as well) appeared not to have any in-depth training in lipid chemistry or biochemistry. Now after some 37 years, the concern is the same but the pendulum has swung in other directions. There has been the dramatic emergence of molecular biology and its implied goal of cure of all diseases through cloning and gene manipulation, along with the revolutionary changes in our understanding of membrane biochemistry and biology. Certainly the latter is an exciting area in a personal sense because it focuses attention on the behavior of membrane lipids (primarily phosphoglycerides) and now shows that these lipids are important components of the signal transduction system initiated when an agonist interacts with a sensitive cell. Even a casual glance at most biochemically oriented journals will show an enormous number of publications in which many different methods are used to explore changes in membrane lipids subsequent to the agonist interaction. However, in reading the literature it is my personal feeling that many investigators do not really have a basic understanding of the nuances of lipid chemistry and biochemistry. In a way, that is understandable because very few major university settings (main campus, medical school, etc.) have faculty with an expertise in the area of lipid

chemistry and biochemistry. There are probably less than ten major universities in this country in which graduate courses in the chemistry and biochemistry of lipids per se are offered. Essentially we are at the nadir of training of persons with a true expertise and/or knowledge in lipids and yet we have an explosive interest in these compounds in biological reactions. A perplexing situation which one hopes will change to the better in the future! Thus a large majority of students emerging in the biological sciences are never exposed to this discipline.

The primary goal of this book is to provide a gentle introduction to phospholipid chemistry, particularly as it might be applied to a study of these compounds in mammalian cells. Inasmuch as the authors' research interest over the past several years has been intimately involved with the role of phospholipids in the signal transduction process using rabbit or human platelets as a model cell, most experimental examples center on this cell. The first three chapters are devoted to an orientation or introduction to the chemical nature of lipids in general, how they are thought to be associated in the cell, and the methodology by which the cellular lipids including the phospholipids can be recovered from cells and subjected to an initial identification. Then the subsequent chapters are concerned with the chemical characteristics and characterization of the choline-containing phospholipids, including the sphingophospholipids and then the major non-choline-containing phospholipids and finally the so-called minor phospholipids. These latter compounds, though low in concentration in cells, are very high in visibility because they form the vanguard of a new category of biologically active substances. As shall be mentioned often in the main text, the finding of biologically active phospholipids (that act as agonists or lipid chemical mediators on cells) has set the stage for the study of cellular phospholipids on a new and exciting course. Finally it should be emphasized that this book is intended to provide a basis for further inquiry by an individual investigator on these complicated molecules and to show that these compounds are unique but, with care and understanding, can be studied with ease. The text follows in general the format of a graduate course given to first- and second-year graduate students in our Ph.D. degree track here. In any event it is hoped that this book will be of benefit to graduate students, postdoctoral fellows, and interested senior investigators.

The author wishes to acknowledge the generous and continued support of Dr. Merle S. Olson during the lengthy birth of this book. A debt of gratitude is owed to Esther L. Hall, whose wise, compassionate, and dedicated help brought this book to fruition.

San Antonio, Texas

D.J.H.

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INTRODUCTION TO LIPIDS

Prologue

Concomitant with the explosive development and progress made in the field of molecular biology, initiated by development and proof of the double helix structure of DNA some 40 years ago, there has been a much more subdued, but equally exciting and important, development in the area of signal transduction (Nishizuka, 1992; Berridge, 1984; Exton, 1990). What makes the latter subject so enchanting to biochemists is the finding that membrane phospholipids are intimately involved in the transduction process. Interestingly, the potential role of phospholipids in the signal transduction pathway was formulated some 40 years ago also. (Perhaps all great discoveries occur in 40 year cycles.) In any event the first hint of any possible involvement of phospholipids in cellular stimulus responses was gained from the work of Hokin and Hokin, which first appeared in 1953. In this classic paper, these investigators reported that treatment of pigeon pancreas slices with acetylcholine or carbamylcholine (cholinergic drugs) resulted not only in the secretion of amylases but also in the turnover of two specific membrane phospholipids, namely, phosphatidylinositol and phosphatidic acid. While the entire process of stimulus response in a mammalian cell is now much more complicated, nevertheless the findings by Hokin and Hokin were of major importance in the maturation of this field.

Unfortunately, the impact of the Hokins' observations was not immediately felt. At that point in time, phospholipids were viewed mainly as semipermeable membrane structures whose main function was to regulate the ion content of the cell. In addition, another deterrent was the limited information on the chemical structure of the mammalian cell phospholipids. Hence

there was a hiatus of many years in which low-profile lipid chemists and biochemists labored to solve the chemical nature of membrane lipids and to deduce their physical arrangement in the cell. Then in 1975, Michell published a key paper (Michell, 1975) in which he noted the importance of the inositol-containing phospholipids in the membrane process known as *calcium gating*. This paper initiated what can be called the "PI" era, which is still very much alive and well today.

This brief historical sketch serves as an introduction to the main goal of this book, which is to describe in some detail the chemical nature of phospholipids present in mammalian cells. It is hoped that there will be sufficient information for the reader to appreciate the uniqueness of many of these compounds, develop some rapport with their chemical structure, and become familiar with their isolation and identification.

This book will not be encyclopedic in nature and will not approach the status of a tome but rather will provide a gentle introduction to a fascinating group of compounds. Prior to a discussion of specific phospholipids, it seems worthwhile to provide some general background, definitions, and approaches used in obtaining these lipids from a cell. First, it is appropriate to consider some general facts about lipids per se.

Lipids: What Are They?

Lipids are a complex group of substances, which include the long-chain fatty acids and their derivatives, sterols and steroids, carotenoids, and other related isoprenoids. It is evident that the term *lipid* denotes a wide range of compounds that appear to have little obvious interrelation. However, although these compounds possess widely different structures, they are derived in part from similar biological precursors and exhibit similar physical and chemical characteristics. Furthermore, most lipids occur naturally in close association with protein, either in membranes as insoluble lipid-protein complexes or as soluble lipoproteins of the plasma.

Some of the lipids are of considerable importance as energy sources. Hence, oxidation of long-chain fatty acids, stored in the adipose tissue of mammals as triacylglycerols (triglycerides), may supply as much as 80% to 90% of the total energy requirements under certain circumstances. The close relationship of this metabolic path to that of carbohydrate utilization is well established. Independent of their use as fuels, the group of biologically active lipids includes the steroid hormones (estrogens, androgens, and adrenocortical hormones), other sterols (bile acids, vitamin D), and various terpenoids [β -carotene, retinal (vitamin A aldehyde)], all of which are essential components of specific metabolic processes.

In any attempt to evaluate adequately the activity or role of lipids in biological systems, it is of prime importance to acquire a firm understanding of their chemistry. This discussion will emphasize the chemical characteristics

must clearly include an in-depth understanding of the chemistry of these two important classes of compounds.

Thus, it is the intent to limit attention here to the phospholipids and to their interrelation with other components of membranes and with each other; examples of their participation in biologically important reactions will be explored. Prior to an in-depth treatment of the chemistry of the phospholipids, it seemed appropriate to describe some general facets of their biochemistry, especially with regard to approaches to isolation, purification, structure proof, and so on. In addition, it is appropriate to include a very brief resume of the types of fatty acids commonly found in naturally occurring lipids because it will complement later discussions on the complex phospholipids.

Fatty Acids: Very Vital Constituents

On a purely quantitative basis there can be no doubt that the fatty acids are one of the important constituents of the lipids of all living cells. The term *fatty acid* is applied to the monobasic carboxylic acids of the general formula



where R may be a straight-chain saturated (alkyl) or an unsaturated (alkenyl) hydrocarbon residue; in the more prevalent naturally occurring fatty acids the R group may vary in chain length from C₁₁ to C₂₃ and may contain in addition hydroxy, keto, branched, or even alicyclic substituents. Inasmuch as it is not possible to consider here in depth all of the naturally occurring fatty acids, emphasis will be placed on the more commonly encountered types.

A more detailed description of the fatty acids may be gained from reference sources (Markeley, 1960, 1961, 1964; Kuksis, 1978). It should be emphasized, however, that fatty acids, in the free form, exist in very low levels, with the main forms being carboxylic esters or amides.

Saturated

The most representative saturated fatty acids found in animal, plant, and, to a lesser extent, bacterial lipids are palmitic acid and stearic acid (Figure 1-2).

The systematic names for these acids are given in parentheses; the systematic terms are preferable to the trivial names when it is necessary to describe the geometric isomerism of an unsaturated fatty acid or the exact location of a substituent group.

Palmitic and stearic acids are the major saturated fatty acid constituents of most animal and plant tissues. Much smaller amounts of other saturated fatty acids are present in most natural sources. Low concentrations of myristic acid (*n*-tetradecanoic acid; 14:0) and lauric acid (*n*-dodecanoic acid; 12:0) have been detected in certain tissues.

Higher fatty acids such as lignoceric acid (24:0) and behenic acid (22:0) are found in high concentrations in brain sphingolipids. Finally, evidence exists for

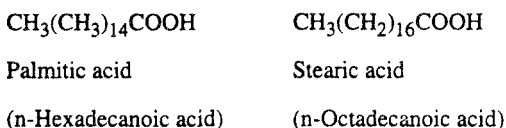


FIGURE 1-2. Long-chain saturated fatty acids found in mammalian cells.

the occurrence of fatty acids with an odd number of carbon atoms, such as pentadecanoic acid (15:0) and heptadecanoic acid (17:0); however, these latter acids are present in very low concentrations.

A list of the more common saturated straight-chain fatty acids, which gives their usual sources, is presented in Table 1-1.

It is important to emphasize that lower homologs of these and other fatty acids are also of biological significance. Specifically, the shorter-chain saturated fatty acids [e.g., butyric acid (4:0) and caproic acid (6:0)], are important constituents of milk lipids, and octanoic acid (8:0) and decanoic acid (10:0) are present in high concentrations in palm oil. The short-chain fatty acids are rarely found in mammalian organs or tissues—with the exception of milk, where they are in relatively high concentration.

Unsaturated

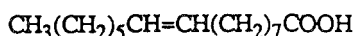
These long-chain fatty acids have formulae similar to those described above but contain one or more double bonds. This group of fatty acids may be subdivided into monoenoic and polyenoic types. A general summary of the chemical nature of the unsaturated fatty acids follows.

MONOENOIC. (Monoethenoic, Monounsaturated, Ethylenic, Alkenoic). Although acids containing olefinic bonds (double bonds) or acetylenic bonds (triple bonds) have been isolated from natural sources, most attention will be directed toward the former types.

The commonly occurring unsaturated fatty acids contain a single double bond. There are a large number of possible monoenoic acids; not only are

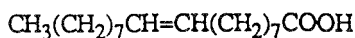
TABLE 1-1. Some of the More Common Saturated Straight-Chain Fatty Acids

Trivial Name	Systematic Name	Chain Length in Carbon Atoms	Typical Source
Lauric acid	<i>n</i> -Dodecanoic acid	12	Palmkernel oil, nutmeg
Myristic acid	<i>n</i> -Tetradecanoic acid	14	Palmkernel oil, nutmeg
Palmitic acid	<i>n</i> -Hexadecanoic acid	16	Olive oil, animal fat
Stearic acid	<i>n</i> -Octadecanoic acid	18	Cocoa butter, animal, fat
Behenic acid	<i>n</i> -Docosanoic acid	22	Brain, radish oil
Lignoceric acid	<i>n</i> -Tetracosanoic acid	24	Brain, carnauba wax



Palmitoleic acid

(*cis*-9-Hexadecenoic acid)



Oleic acid

(*cis*-9-Octadecenoic acid)

FIGURE 1-3. Representative long-chain monounsaturated fatty acids.

there many possible positions for the olefinic bond, but geometric isomers can also occur. These considerations plus the fact that fatty acids of different chain lengths exist make the problem appear immense. However, relatively few of the theoretically possible monoenoic acids occur in animal and plant tissues. Thus, the major and most representative monoenoic acids occur in animal and plant tissues. The major and most representative monoenoic acids present in animal and plant tissue are oleic acid and palmitoleic acid (Figure 1-3).

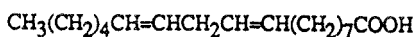
The structures of these fatty acids are characterized by (a) the presence of an olefinic bond between carbon atoms 9 and 10 (counting from the carboxyl end) and (b) the occurrence of a *cis* configuration rather than a *trans* configuration at the double bond. Fatty acids are known in which a single double bond occurs at other positions and in which a *trans* configuration is present, but these are relatively rare.

A list of the common monoenoic fatty acids is given in Table 1-2.

POLYENOIC. [Polyethenoic, Polyunsaturated, Alkapolyenoic (Alkadienoic, Alkatrienoic, etc.)]. In contrast to the monounsaturated fatty acids, these unsaturated fatty acids are found in much smaller amounts in naturally occurring lipids. The structural formulas of the three most commonly encountered polyunsaturated fatty acids are in Figure 1-4. Linoleic acid and arachidonic

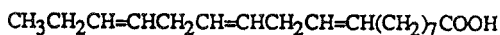
TABLE 1-2. Common Monoenoic Fatty Acids

Trivial Name	Systematic Name	Chain Length in Carbon Atoms	Typical Source
Palmitoleic acid	<i>cis</i> -9-Hexadecenoic acid	16	Marine algae, pine oil
Oleic acid	<i>cis</i> -9-Octadecenoic acid	18	Animal tissue, olive oil
Gadolcic acid	<i>cis</i> -9-Eicosenoic acid	20	Fish oils (cod, sardine)
Erucic acid	<i>cis</i> -13-Docosenoic acid	22	Rapeseed oil
Nervonic acid	<i>cis</i> -15-Tetracosenoic acid	24	Elasmobranch fish, brain



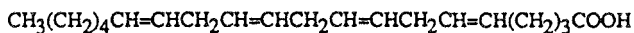
Linoleic acid

(cis-9, cis-12-octadecadienic acid)



Linolenic acid

(cis-9, cis-12, cis-15-octadecatrienoic acid)



Arachidonic acid

(cis-5, cis-8, cis-11, cis-14 eicosatetraenoic acid)

FIGURE 1-4. Primary long-chain polyunsaturated fatty acids.

acid form the bulk of the polyunsaturated fatty acids in most animal tissues. As evident from the structures given in Figure 1-4, these acids possess more than one center of geometric isomerism (as the double bonds) and hence many isomeric forms are possible. An examination of the polyunsaturated fatty acids found in nature shows that there is a marked prevalence of acids possessing the following characteristics: (a) nonconjugated double bonds (frequently termed a divinylmethane rhythm, as in linoleic acid); (b) double bonds at specific positions, as in linoleic and arachidonic acids; and (c) *cis* configuration at all the double bonds.

A series of the more frequently encountered and relatively important polyunsaturated fatty acids is listed in Table 1-3.

TABLE 1-3. Series of the More Frequently Encountered and Relatively Important Polyunsaturated Fatty Acids

Trivial Name	Systematic Name	Chain Length in Carbon Atoms	Typical Source
Linoleic acid	<i>cis</i> -9, <i>cis</i> -12-Octadecadienoic acid	18	Corn oil, animal tissue, bacteria
Linolenic acid	<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15-Octadecatrienoic acid	18	Animal tissues
—	5,8,11-Eicosatrienoic acid	20	
—	8,11,14-Docosatrienoic acid	20	Brain
—	7,10,13-Docosatrienoic acid	22	Phospholipids
—	8,11,14-Docosatrienoic acid	22	
Arachidonic acid	5,8,11,14-Eicosatetraenoic acid	20	Liver, brain
—	4,7,10,13-Docosatetraenoic acid	22	Brain
—	4,7,10,13,16,19-Docosahexaenoic acid	22	Brain

It is noteworthy that virtually all of the naturally occurring polyunsaturated fatty acids contain 18–22 carbon atoms. Linoleic acid is the predominant polyunsaturated fatty acid. It is of considerable interest that most animals cannot synthesize linoleic acid and must take it in the diet. If insufficient amounts of this acid are present in the diet of animals, severe symptoms, such as skin lesions, kidney damage, cataracts, increased permeability to water, and so on, can occur. Thus, the term *essential fatty acids* has been applied to these compounds. It is not certain, however, that dietary unsaturated fatty acids are needed by the human adult, but there is evidence of such a requirement by the human infant.

Summary

This brief excursion into the chemical nature of naturally occurring fatty acids should attest to their diverse structural characteristics. In general, the aforementioned fatty acids occur only to a limited extent in the free form but occur more frequently in the ester or amide form. The possible number of permutations in fatty-acid-containing species are impressive and provides an interesting and challenging problem. Certain fatty acids tend to be identified rather characteristically with specific lipids, namely, phosphoglycerides and sphingolipids. Furthermore, a close examination of the positioning of fatty acids on certain phosphoglycerides reveals a high degree of specificity. Thus, most mammalian tissues—phosphatidyl choline, for example—contains primarily saturated fatty acids on the C-1 position and primarily unsaturated fatty acids on the C-2 position. Other phospholipids tend to have similar structural patterns with regard to positioning of fatty acids or in the specific types of fatty acids present.

A Comment on Nomenclature

Phosphoglyceride refers to a glycerophosphoric acid derivative that contains a minimum of one *O*-acyl, *O*-alkyl, or *O*-alk-1-enyl group covalently linked to the glycerol backbone. An example would be 1,2-diacyl-*sn*-glycero-3-phosphoric acid, which is often referred to in the literature as *phosphatidic acid*. The latter (with one less hydrogen on the phosphate group) is frequently called a *phosphatidyl group*, which is used to define, for example, a derivative with a choline group esterified to the phosphoric acid moiety on a phosphatidic acid. Hence the derivation of the term *phosphatidylcholine*. The use of this type of terminology is widespread and is very convenient. Of course, if the configuration is known, one could use the term 3-*sn*-phosphatidylcholine or 1,2-diacyl-*sn*-glycero-3-phosphocholine.

Phospholipid is a generic term that refers to lipids containing a phosphoric acid residue. Thus, both phosphatidylcholine and sphingomyelin would fit in this category.

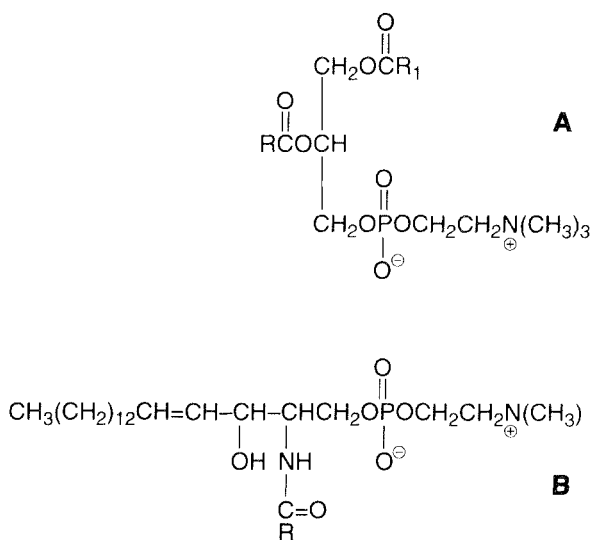


FIGURE 1-5. Two phospholipids commonly encountered in the cellular membranes.

Some Basic Facets of Phospholipid Structure

The More Prevalent Forms

It is appropriate at this point to provide a general description of the types of phospholipids one encounters in mammalian cells and those which we will be considering in this book. Essentially there are two major classes of phospholipids, one of which contains a glycerol backbone and the other of which contains a sphingosine backbone. Their general structural formulae are presented in Figure 1-5.

Structural formula A is typical of a group of compounds commonly called phosphoglycerides. In this particular depiction the symbols R_1 and R_2 are long-chain carboxylic acids linked in an ester linkage to the primary and secondary alcohol residues of glycerol. The symbol X is a mixed venue with a wide variety of bases—for example, choline ($\text{HOCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3$) or

OH

ethanolamine ($\text{HOCH}_2\text{CH}_2\text{NH}_2$)—joined in ester linkage to the phosphoric acid residue.

As we shall see later, nature, as expected, is not so simple and straightforward, since variation of the structure given in A can occur. Thus a vinyl ether ($\text{O}-\text{CH}=\text{CH}-\text{R}$) residue or a saturated ether moiety ($\text{O}-\text{CH}_2\text{CH}_2\text{R}$) can replace the carboxylic acid ester on carbon 1 of glycerol. In addition, there can be at least eight different substituents associated with the phosphoric acid

residue. Thus the number of permutations in structure can be enormous, perhaps as great as 1000, given variability in the substituent at the C-1 and C-2 positions.

However, one should not despair at this complexity since there is some order in all this chaos. First only one type of backbone residue, glycerol, is found in the most prevalent type of phospholipids, the phosphoglycerides. Even the sphingophospholipids (formula B) in mammals have only one other type (minor) of backbone group, a saturated form called *dihydrosphingosine*. Second, high stereospecificity is exhibited by the phosphoglycerides, with only one form (the *sn*-3 configuration) being found (stereospecificity will be discussed again later in this chapter). Positional specificity also predominates in the phosphoglycerides, with the C-1 position containing primarily (over 95%) saturated residues and the C-2 position having over 95% unsaturated residues. Finally, the phosphoric acid residues are found mainly (over 98%) at the C-3 alcohol position. Thus, it is rather comforting to see that there are some common denominators among these complex lipids.

Structure B shows the chemical formula of a sphingosine-based phospholipid, sphingomyelin. This compound is the most predominant form found in mammalian tissues and possesses some interesting chemical features. The backbone, in this case a long-chain amino alcohol (sphingosine), is normally found with the amino function amidated with a long-chain fatty acyl chain and with a phosphoric acid group on the alcohol group. In addition, the latter function can be esterified to a base such as choline (a prevalent form). Only a minor amount of a saturated form, dihydrosphingosine, has been detected. Some evidence for a longer-chain hydrocarbon has been presented, but again these forms are normally very minor constituents of mammalian cells. Interestingly, while there are many different chain lengths (and degrees of unsaturation) noted with the fatty acids associated with the phosphoglycerides, there are relatively few different types of fatty acids found in the sphingomyelins; these are mainly saturated with restricted chain lengths (e.g., 18:0 and 22:0). Further discussion of these compounds and more complicated 18:0 and 22:0 forms, such as ganglioside, will occur in Chapter 4.

Stereochemistry of the Phosphoglycerides

All configurational assignments rest upon the classic work of Baer and his associates. They used the D/L system, which placed monoacylglycerol in the same category as the glyceraldehyde into which it could be transformed by oxidation, without alterations or removal of any substituent (Baer and Buchnea, 1959). Steric representation of a triglyceride is thus possible as shown in Figure 1-6. Equivalent but easier to show is the structure depicted in Figure 1-7.

While an optical classification of triacylglycerols could be made, confusion arose over the two primary alcohols.