LECITHINS
Sources, Manufacture & Uses

Edited by

Bernard F. Szuhaj

American Oil Chemists' Society
Champaign, Illinois

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Library of Congress Cataloging-in-publication Data

Lecithins: sources, manufacture & uses / edited by Bernard F. Szuhaj.
p. cm. — (AOCS monograph; 12)
Includes bibliographies and index.
ISBN 0-935315-27-6
1. Lecithin. I. Szuhaj, Bernard F. II. Series.
TP453.L43L44 1989
661'.8—dc20
89-6769
CIP

Printed in the United States of America.

Foreword

Lecithin is a widely used commercial term for a complex mixture of phosphatides produced from a variety of vegetable and animal sources. Natural phosphatides have innumerable uses, yet limited volume.

In order to maximize the sources and volume, the AOCS Education Committee and Timothy Mounts decided at this time it would be beneficial to have a short course on lecithin emphasizing the importance of this minor component, yet major natural product in functional applications.

A program comprising twenty experts was organized to precede the National Meeting in Phoenix, Arizona, to meet this challenge. This short course resulted in a clearer understanding of the nomenclature, sources and applications of phospholipids in the food, industrial, and pharmaceutical areas.

The AOCS Monograph 12 on *Lecithins* was the first attempt since Wittcoff's book on *Phosphatides* to illustrate the world of lecithin to the scientific and engineering community. This book is a compilation of the lectures presented at this short course. It should be a valuable collection to the participants and others who want to know more about Lecithins: Sources, Manufacture, and Uses.

Bernard F. Szuhaj,
Chairman, AOCS Lecithin Short Course
Phoenix, Arizona, May 4-7, 1988

Acknowledgments

The Chairman and Editor wish to thank the Faculty for their participation in this short course, for their time and devotion to their subjects, and especially their timely manuscript submission.

A special thanks goes to Endre F. Sipos who took the responsibility of chairing the second and third days program. Thanks also goes to Joan Dixon and the AOCS support staff who helped put the program together at The Pointe at Squaw Peak.

Finally, I want to thank Barbara M. Gillman, Lecithin Short Course Coordinator, who devoted many hours pulling the program together, keeping track of the Faculty and their manuscripts, and making the Chairman's job a lot easier and this book possible.

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Chapter One

Nomenclature and Structure of Phosphatides

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The major components of biological tissues were classified as glucides (carbohydrates), protides (proteins), and lipides (lipids) many years ago. Fats and fat-like substances were also known as lipoids and lipins. The latter term was used originally for complex lipids (1). The term phosphatides was assigned by Thudichum (2) to lipids containing phosphorus. The classification of lipids according to Bloor and Deuel is as follows (3,4):

- Simple Lipids
  - A. Neutral Fats
  - B. Waxes
- Compound or Conjugate Lipids
  - A. Phospholipids
    - 1. Lecithin
    - 2. Cephalin
      - a. Phosphatidylethanolamine
      - b. Phosphatidylserine
      - c. Phosphatidylinositol
    - 3. Sphingomyelin
    - 4. Phosphatidic Acid
  - B. Cerebrosides
  - C. Sulfolipids
- Derived Lipids
  - A. Fatty Acids
  - B. Alcohols
    - 1. Straight-chain Alcohols
    - 2. Sterols
    - 3. Vitamin A and Carotenols
  - C. Hydrocarbon
    - 1. Aliphatic Hydrocarbons

        2. Carotenoids
        3. Squalene
    D. Vitamin D
    E. Vitamin E
    F. Vitamin K

Egg yolk and brain were the sources (5,6) of a lipid containing phosphorus that was called lecithin (7) after the Greek word for egg-yolk and contained glycerophosphoric acid. The presence of choline (from the Greek word for bile) esterified to phosphate was established in 1868 (8). Thudichum (9) separated cephalin (from the Greek word for head) from brain lipids by its relative insolubility in warm ethanol. Sphingomyelin was recognized by its separation from warm ethanol on cooling and content of sphingosine, choline, phosphoric acid, and a fatty acid (9). Differences in solubilities were utilized to separate cephalin into three fractions containing mainly ethanolamine, serine, and inositol glycerophospholipids (10,11). Plasmalogens were found in the ethanolamine glycerophospholipid fraction (12,13), but the presence of a fatty acid was not shown. The correct structure of the ethanolamine plasmalogen was not proven until 1959 (14-16).

The structure of phosphatidylcholine (lecithin) was established by chemical synthesis using 1,2-distearoyl-*sn*-glycerol and phosphoric acid anhydride to form the metaphosphoric acid ester followed by treatment with choline bicarbonate to form the bicarbonate salt of phosphatidylcholine (17,18). The acyl groups at the 2-position had been modified earlier by hydrolysis with cobra venom followed by reacylation (19). The 2-lyso phosphatidylcholine was called lysocithin (20). Purified preparations from beef brain and egg yolk were made with water moccasin venom (21).

The Nomenclature Commission of the International Union of Biochemistry and the International Union of Pure and Applied Chemistry issued Tentative Rules for the naming of lipids in 1976. These were published in *Lipids* (22).

The Commission discourages use of the old terms monoglyceride, diglyceride, and triglyceride in favor of monoacylglycerol, diacylglycerol, and triacylglycerol, respectively, because the latter convey the intended meaning accurately. Since a glyceride is a compound of glycerol, triglyceride could be understood as a trimer of a glyceride (a compound with 3 glycerols). Phospholipid is defined as any lipid containing phosphoric acid. A glycerophospholipid is any derivative of glycerophosphoric acid that contains at least one 0-acyl, 0-alk-1-enyl, or 0-1-alkyl group attached to the glycerol residue. The old terms phosphatide and phospho-glyceride are no longer recommended because they do not convey the intended meaning.

The stereospecific nomenclature of glycerophospholipids places the phosphate at the *sn*-3 position. The *sn* is an abbreviation of stereospecific numbering. Phosphatidyl, the radical of phosphatidic acid, is 1,2-diacyl-*sn*-glycero-3-phospho (Ptd). Thus phosphatidylcholine, a major component of soya lecithin, is 1,2-diacyl-*sn*-glycero-3-phosphocholine (PtdCho). The Nomenclature Commission has defined a number of three-letter abbreviations for common biochemical compounds (Table 1-1). Thus, *sn*-glycero-3-phosphocholine is a Gro*P*Cho. Three-number abbreviations for fatty acids were also proposed by the Commission (Table 1-2) but are not used very often. An example would be the 16:0 - 20:4 (5,8,11,14) specie of phosphatidylcholine, 1-Pam-2-Δ_4Ach-Gro*P*Cho. The majority of publications in scientific journals utilize ambiguous abbreviations for glycerophospholipids and related compounds. For example, PC is used for PtdCho. PC is literally an impossible compound of phosphorus and carbon. PC could also be *P*Cho, a hydrolytic product of PtdCho.

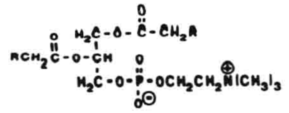
Table 1-1

Symbols for Some Constituents of Lipids

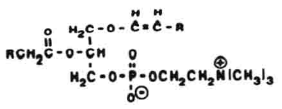
Cho	Choline
Etn	Ethanolamine
Ins	Inositol
Ser	Serine
Gro	Glycerol
Sph	Sphingosine
P	Phosphoric Residue

PC is also often used for the mixture of choline glycerophospholipids that is obtained by chromatography. The mixture often contains 1,2-diacyl-Gro*P*Cho, 1-alk-1′enyl-2-acyl-Gro*P*Cho, and 1-alkly-2-acylGro*P*Cho (Fig. 1-1). This mixture is correctly described as choline glycerophospholipids, ChoGpl, or 1,2-diradyl-Gro*P*Cho and contains PtdCho, PlsCho, and PakCho, respectively. Radyl is any hydrocarbon side chain such as acyl, alk-1-enyl, or alkyl.

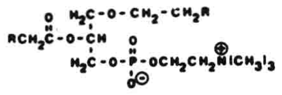
Catalogs of biochemical suppliers and older publications often contain the nomenclature that preceded stereospecific numbering. Thus, natural PtdCho is L-α-phosphatidylcholine (L-α-lecithin), and the diacylglycerol obtained with a phospholipase C is the D-2,3-diglyceride.



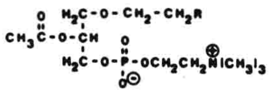
1,2-diacyl-*sn*-Gro-3-*P*Cho
(phosphatidylcholine, PtdCho)



1-alk-1′-enyl-2-acyl-*sn*-Gro-3-*P*Cho
(plasmenylcholine, PlsCho)



1-alkyl-2-acyl-*sn*-Gro-3-*P*Cho
(alkylacyl-Gro*P*Cho, PakCho)



1-alkyl-2-acetyl-*sn*-Gro-3-*P*Cho
(platelet activating factor)

Fig. 1-1. Structures and nomenclature of choline glycerophospholipids. Ptd is an official abbreviation. Pls is proposed as an abbreviation for plasmenyl, an official term derived from the common name plasmalogen. Pak is proposed as an abbreviation for phosphalkanyl, a substitute for the official term. PakCho includes platelet activating factor, 1-alkyl-2-acetyl-Gro*P*Cho, a potent hypotensive agent and activator of platelet aggregation at nanomolar concentrations.

TABLE 1-2

Symbols for Some Acyl Groups

Myr	14:0
Pam	16:0
Ste	18:0
Ole	18:1 (9)
Lin	18:2 (9,12)
αLnn	18:3 (9,12,15)
Δ_4Ach	20:4 (5,8,11,14)
Ner	24:1 (15)

Synthetic examples are L-α-lecithin, β,γ-dioleoyl and L-α-lecithin, β, γ-dihexadecyl. The latter is not a phosphatidylcholine but is 1,2-dihexadecyl-Gro*P*Cho, an analog of PakCho with two alkyl groups.

Commercially available synthesized phosphatidylcholines usually contain the same fatty acid at both the *sn*-1 and *sn*-2 positions. Biochemical supply catalogs and scientific papers often refer to these as derivatives of phosphatidylcholine, for example, dipalmitoyl phosphatidylcholine. Although it is generally understood that dipalmitoyl refers to a part of the phosphatidylcholine, the term is ambiguous because phosphatidylcholine already includes two acyl groups. Dipalmitoyl phosphatidylcholine implies two additional acyl groups for a total of four. The solution is to change the phosphatidylcholine to glycerophosphocholine in order to specify the compound more exactly. DPPC is a common abbreviation for dipalmitoyl phosphatidylcholine. Each P in this abbreviation has a different meaning, namely palmitoyl and phosphatidyl. Good abbreviations should be understandable and unambiguous, so this abbreviation is particularly bad. The shortest acceptable abbreviations are $Pam_2GroPCho$ or Pam_2GPC. The latter is shorter but is dependent on being defined when used.

Soya lecithin contains $acyl_3Gro$, PtdEtn, PtdIns, and other compounds in addition to PtdCho. Egg lecithin contains some PakCho together with PtdCho and other compounds depending on the grade. The term "lecithin" should be reserved for such industrial products. Cephalin was abandoned years ago because it refers to a mixture of compounds. Chemists and biochemists should now also abandon lecithin as a synonym for phosphatidylcholine or choline glycerophospholipids because the latter terms are more precise and descriptive. The components of lecithin products are often described with the terms "chemical lecithin" and "chemical cephalin". These should be abandoned in favor of phosphatidylcholine and phosphatidylethanolamine because the latter terms are more descriptive and have the easily understood abbreviations PtdCho and PtdEtn.

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Chapter Two

The Chemistry and Reactivity of the Phosphatides

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The chemistry of the phosphatides is generally that of their ester linkages, unsaturated fatty acids and other reactive groups. As Pryde (1) stated in his review of phosphatide chemistry, the chemical reactions of phosphatides provide few surprises. The approximate structures of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), then called lecithin and cephalin, were established by early workers. Together with phosphatidylinositol (PI), they are the most abundant phosphatides in many mixtures (2). Most of the applicable reactions of organic chemistry have been employed in their study. Some reviews of phosphatide chemistry are listed (1-6).

Hydrolysis

The first effect of either acid or alkaline hydrolysis is the removal of the fatty acids. Brockerhoff (7) found that for minimal breakdown of water soluble phosphate diesters, deacylation should be carried out at moderate base concentrations in a highly polar solvent. He achieved less than 0.1 percent breakdown of water soluble diesters from most lipid preparations in 0.125 M lithium hydroxide for 15 min.

With continued hydrolysis, the water soluble glycerophosphate diesters break down to a mixture of α-and-β-glycerophosphoric acids (GPA). The optically active α-glycerophosphoric acid is at least partly racemized. Acid hydrolysis of glycerophosphoric acid and diesters such as glycerylphosphorylcholine (GPC) yield about 75% α- and 25% β-glycerophosphoric acid. In alkali, phosphatides and diesters yield

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approximately equal amounts of α- and β-glycerophosphoric acids. However, in alkali, free glycerophosphoric acid and the glycerophosphoric group in phosphatidic acids are not rearranged.

Alkaline hydrolysis is much more rapid than acidic. Baer and Kates (8) found in 1N NaOH at 37°C that one half the choline from GPC was liberated in 0.9 hrs, and the reaction was complete in 7 hrs. The corresponding times in 1N HCl were 10 and 20 hrs.

Verkade *et al.* (9) suggested a cyclic glycerophosphate ester intermediate to explain the interconversion of α- and β-glycerophosphoric acids. Baer *et al.* (10,11), Long and Maguire (12), Maruo and Benson (13), and DeKonig and McMullan (14) have studied the hydrolysis of natural and synthetic phospholipids, glycerylphosphoryl esters and other model compounds. They suggested mechanisms involving cyclic ester intermediates. Such a cyclic glycerophosphoric acid has been synthesized by Ukita *et al.* (15). A scheme proposed by Baer and Kates for acid hydrolysis of glycerylphosphorylcholine follows (8).

L-α-GPC ⇄ cyclic L-GPC
cyclic L-GPC → β-GPC
β-GBC ⇄ cyclic DL-GPC
cyclic DL-GPC ⇄ DL-α-GPC

All substances can lose choline and form glycerophosphoric acid.

L-α-GPA ⇄ cyclic L-GA → β-GPA
β-GPA ⇄ cyclic DL-GPA ⇄ DL-α-GPA

For alkaline hydrolysis, Long and Maguire (12) suggest that the loss of choline takes place directly from the cyclic ester. Brown *et al.* (16) explain the lack of interconversion in alkali of the free acids by suggesting a cyclic transition state rather than intermediate.

Glycerylphosphorylcholine and glycerylphosphorylethanolamine (GPE) are insoluble in ether and may be isolated as solids after alkaline hydrolysis of phosphatides in ether solution (17,18).

Maruo and Benson (13) found PI more resistant to cyclic ester formation during alkaline methanolysis. Dawson (19) found 57% glycerylphosphorylinositol and 14% phosphoryl inositol after hydrolysis with sodium hydroxide at 37°C for 20 minutes, a procedure that produced mostly glycerylphosphoryl esters with other phosphatides. A cyclic 1,2 inositol phosphatate may be formed in a manner analogous to the cyclic glycerophosphate. Pizer and Ballou (20) found 80-90% inositol-1-phosphate and 10-20% inositol-2-phosphate after alkaline hydrolysis of soybean PI. Hawthorne (21) also discusses the formation of a cyclic inositol phosphate intermediate and cites several workers who found 31-40% inositol after hydrolysis of glycerylphosphorylinositols.

Renkonen (22) noted that although the common diacyl phosphatides were readily deacylated to yield water soluble phosphorus, the phosphorus of plasmalogens and alkoxy phosphatides remained ether soluble. A combination of mild alkali and acid hydrolysis produced water soluble phosphorus from plasmalogens but not from alkyl ether phospholipids.

Acetolysis

Renkonen (23) dephosphorylated phosphatides with an acetic anhydride, acetic acid 2:3 mixture at 145°C; 1,2 diglyceride acetates were formed from PC, PE, PI and phosphatidylserine (PS), but the fatty acids moved from their original positions (24,25). Therefore, the reaction cannot be used to determine the position of fatty acids in phosphatides. Treatment with acetic anhydride at 60°C for 15 min. has also been used in refining oils (26). The lecithin has properties similar to that prepared by usual procedures so dephosphorylation under these conditions must be minimal.

Hydrogenolysis

Egg lecithin has been reduced with $LiAlH_4$ (molar ration 1:2) in ether solution to yield 95% glycerylphosphorylcholine (27,28). With larger excesses of $LiAlH_4$, the phosphate moity is partly reduced, and 50% choline is found (29). Under more severe conditions, the phosphate is completely reduced, and the reaction has been used to decompose diacyl phospholipids and leave the ether portions of plasmalogens and glyceryl ether phospholipids (30,31). However, $LiAlH_4$ has been shown to give low yields of 0-alk-1-enylglycerols and Vitride, $NaAlH_2(OCH_2CH_2OCH_3)_2$, is a more suitable reagent (32,33).

Egg lecithin has also been reduced by $NaBH_4$. At 37°C, 94% of the acyl ester groups were reduced in 60 min. There was no mention of reactivity of the phosphate group (34).

Thermal Decomposition

Glycerophosphatides lose phosphoric acid to form diglycerides when heated to 250°C in phenyl ether solution (35). A one minute reaction time gives mostly 1,2 diglycerides, and a five minute reaction time produces a larger yield of mostly 1,3 diglycerides. Silyl derivatives of the diglycerides may be analyzed by gas chromatography.