Plant Lipids[1]

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Abstract

The literature on lipid composition of algae and higher plants is reviewed to August, 1964. The complex glycero- and sphingolipids which have been reported from these sources are cataloged, and reference is made to key papers relating to their characterization, structure, and distribution.

Introduction

THE PACE HAS QUICKENED in plant lipid chemistry, so much so, that papers published more than a decade ago are, with notable exceptions, seldom of more than historical interest. Yet most of the significant problems still await solution. The lipid composition of no plant or plant seed is completely known. Proteolipids have been reported, but have not been properly purified and characterized. The number and nature of the complex plant glycolipids are still to be determined, although Carter's laboratory has made progress with these materials. Fruitful consideration of the role of lipids in biological function is only now becoming feasible. It is therefore worthwhile to consider the currently known types of plant lipids, and some of the methodology useful for their isolation. For the most part, only complex glycerolipids and sphingolipids of the higher plants and algae have been included here, and little attention has been given to fatty acid composition. An effort has been made to include the key papers relating to each of the known plant lipids of these two types available by August, 1964. The following paper in this series contains a more detailed discussion of methodology, especially as applied to photosynthetic tissue.

Reviews of interest in plant lipid chemistry appearing within the past few years include articles on complex lipids (1), glycolipids and inositol lipids, (2–4), plant steroids, (5), structure and composition of plastids, (6), and the occurrence of phospholipids (7). Comprehensive treatise on "The Chemical Composition of Natural Fats" (8), "The Lipids" (9), and "The Phosphatides" (10) cover much of the earlier work in their respective areas.

Plant Phosphatides

The early information on this subject is covered in Witcoff's monograph "The Phosphatides," which appeared in 1951 (10). An excellent review by Dittmer (7) covers the literature to 1960.

Levene and Rolf (11) began the first careful study of plant lipids with identification of lecithin in soybean. Commercial "lecithin" or "phosphatide concentrates" from soybean or other oil seeds are complex mixtures. In a typical preparation, cleaned and flaked soybeans are extracted with hexane to remove the oil. Phosphatides are separated by hydrating the oil with hot water, centrifuging, and drying under vacuum. The phosphatides may then be further extracted with acetone. Such material has served as a common source for isolation of a variety of plant lipids for further investigations. In general, seeds with a high oil content have a phospholipid content of 2 to 3 percent while other seeds have about 1 percent.

[1] Paper II in the series "Plant and Chloroplast Lipids."

Phosphatidyl Choline and Phosphatidyl Ethanolamine

These lipids are widely distributed in plants. However, the early determinations of "lecithin" and "cephalin" content based on solvent fractionation of the phospholipids or their divalent metal salts rarely involved pure material. Only in special cases can these techniques be expected to produce pure lipid samples. Neither the improved cadmium salt fractionation procedures of Pangborn (12) nor the solvent fractionation procedure developed by Folch (13) for brain lipids have more than limited application.

Phosphatidyl Serine

This is also widely distributed in the plant kingdom. Plant lipid serine was first identified in lipid hydrolysates of peanuts by Hutt and co-workers in 1950 (14), and soon thereafter in wheat and rye (15). It may be present in all plants, but usually in lower concn than phosphatidyl choline or phosphatidyl ethanolamine.

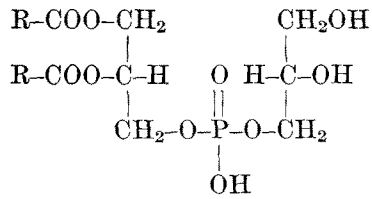
Phosphatidic Acid

The name phosphatidic acid was proposed by Channon and Chibnal for diacylglycerophosphoric acid isolated as the major phospholipid of cabbage (16).

More recent work has shown that phosphatidic acid in high concn is an artifact formed by phospholipase D catalzed hydrolysis of other phospholipids, notably lecithins and cephalins. Cabbage is rich in this enzyme which shows enhanced activity in the presence of calcium ions and ether (17,18), both used in the early isolation of cabbage lipids. Wheeldon (20) has reported a moderate concn of phosphatidic acid as well as an uncharacterized phospholipid in cabbage, but both may be artifacts. Benson and Maruo (19) using tracer techniques did not encounter the uncharacterized lipid but did find a trace of phosphatidic acid in cabbage. A trace is also reported in runner bean leaves (22) but not in *Scenedesmus*, sweet clover, barley leaves, or tobacco leaves (19). On the basis of such evidence it is probable, despite previous suggestions to the contrary (21), that phosphatidic acid does occur in nature. Work not cited in this review indicated that phosphatidic acid is an important metabolic intermediate.

Phosphatidyl Glycerol

This lipid was first detected by Maruo and Benson (23) during investigation of ^{32}P-labeled *Scenedesmus* and *Chlorella* lipid hydrolysates. The structure was partially determined with minute amts of material through tracer chemistry which showed the compound contained a *vic*-glycol and a *vic*-diacylated glycerol (19). Haverkate and co-workers (24), again working with labeled lipid found it was cleaved by phospholipases A, B, C, and D, confirmed the structure proposed by Benson, and suggested that an L-α-configuration was likely on the basis of the phospholipase specificity. The glycerol residues have opposite configurations (25), and the naturally occurring material is optically active (26).

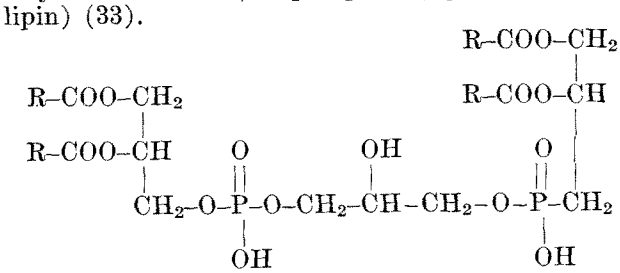


Phosphatidyl glycerol has been isolated from spinach leaves by precipitation of the magnesium salt followed by chromatography on silicic acid columns (27), and by gradient elution from diethylaminoethyl cellulose columns (26). The use of strongly acid solutions during work-up of phosphatidyl glycerol and related phosphodiesters may lead to formation of artifacts since such compounds may be readily cleaved to phosphatidic acid by dilute mineral acid (19).

Phosphatidyl glycerol has been isolated from a number of green plants, and it is probably a universal constituent of photosynthetic tissue (20,27–30). Chloroplasts contain a high concn (31), but it is also present in nonphotosynthesizing tissue (29,32) including corn coleoptile mitochondria.

Other Phosphoglycerolipids

Deacylation products of *Chlorella, Scenedesmus, Rhodospirillum rubrum,* corn, and clover lipids all contain 1,3-diglycerophosphorylglycerol which is probably derived from 1,3-diphosphatidylglycerol (cardiolipin) (33).



Cardiolipin

Nielson cites evidence for the presence of a high mol wt phosphoglycerolipid, possibly a "polyglycerophosphatidic acid," in soybean (34).

Phosphatidyl Inositol and Inositol Glycosides

Lipid-bound inositol in higher plants (soybean) was first reported by Klenk and Sakai in 1939 (35). Subsequently many reports of inositol lipids and inositol glycolipids of plant origin have appeared. These are reviewed by Folch and LeBaron (36), Hawthorne (4), Law (3), and Rapport and Norton (2). Phosphatidyl inositol and to a lesser degree, Carter's phytoglycolipids are the only inositol lipids that have been characterized, but others almost certainly exist. The array of hydrolysis products which have been reported attest to the complexity of the problem, although some of these have surely resulted from phytoglycolipids or are artifacts. Much of the confusion in this area may be the result of the stable Mg-Ca salt complexes formed by pairs of lipids as found by Carter and coworkers (37).

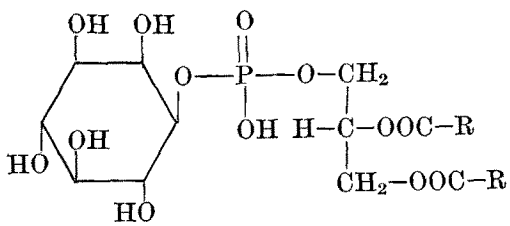
Lipophytin is the name given by Carter to a poorly characterized mixture of inositol polyphosphates obtained by nonpolar countercurrent distribution of several oil seed phosphatides (but not flaxseed) (38). It contains a complex mixture of fatty acids, amines, some glycerol, and a high concn of phosphorus.

Lipositol, a lipid reported to contain tartaric acid (39), was later shown to be a mixture (40,41). The tartaric acid was probably an artifact (36).

An ethanolamine inositol phosphatide isolated from peanut phospholipids as a crystalline material (42) contained glycerol, arabinose, galactose, ethanolamine, and inositol linked as a phosphate. A very tentative structure was proposed, but it seems likely that a mixed lipid was involved.

An acidic inositol phosphatide from soybean (43) was isolated and purified as the Ca-Mg salt. The components (and their approx molar ratios) were nitrogen (1), phosphorus (2), inositol (2), carbohydrate (2), and fatty acid (3). This may also be a mixed lipid complex.

Phosphatidyl inositol has been isolated from a number of plant sources, and is now well characterized. In 1955 Okuhara and Nakayama isolated and identified phosphatidyl inositol from soybean (44), and Ballou and Pizer (45,46) have shown by synthesis that the inositol monophosphate from deacylated soybean or wheat germ phosphatidyl inositol is L-*myo*-inositol-1-phosphate. Phosphatidyl inositol therefore has the structure indicated.

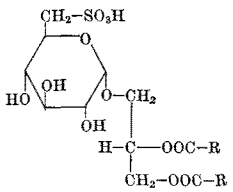


Lepage and co-workers (47) determined that corn phosphatidyl inositol is also 1-phosphatidyl-*myo*-inositol by consideration of optical rotation and rate of hydrolysis of the deacylated lipid. The proton resonance spectrum of the corresponding heptaacetate confirmed the lack of a phosphate ester bond at position 2 of inositol. The phosphoinositol from peanut is also *myo*-inositol-1-phosphate (48). In earlier work Hanahan and Olley (49) showed that phosphatidyl inositol isolated on silicic columns from beef liver, rat liver, and yeast were identical, and demonstrated an O-diglyceride link to phosphoinositol. Faure and Morelec-Coulon developed a procedure for preparation of the sodium salt of phosphatidyl inositol from peanut (50) and wheat germ by solvent fractionation, taking advantage of the stability of the barium salt in diluted hydrochloric acid; the lipid from wheat germ was shown to consist of a glycerophosphatidic acid esterified to inositol (51,52). Inositol phosphates from hydrolysis of soybean, peanut, and ox brain lipids are also chromatographically identical (53). Hörhammer and co-workers (54) found evidence for the identity of phosphatidyl inositol from soybean and bovine tissue through chromatography on formaldehyde treated paper, and reported lysophosphatidyl inositol from both sources (55).

Wagenknecht and Lewin showed that the phosphatidyl inositol content of freshly harvested peas is negligible, rises steadily for several months in cold storage, and then falls off markedly (56). Phosphatidyl inositol was not formed in this manner in blanched or dried peas, as might be expected of an enzymatic process. The Ca-Mg or sodium salts of phosphatidyl inositol can be conveniently isolated from properly aged peas since phytosphingosine-inositol lipids which interfere with isolation from other sources are absent (57). The nature of the phosphatidyl inositol precursor in fresh peas has not been determined. Plasmalogen forms of the phospholipids (especially phosphatidyl inositol) are present (58).

The separation of deacylated phosphatidyl inositols on ion exchange columns (such as Dowex 2, acetate form) has been used for several years (59), but it is only recently that this technique has been made successful for the intact lipids by use of diethylaminoethyl cellulose columns (60). In the separation of brain phosphoinositides Hendrickson and Ballou (61) found that the Ca-Mg salt of triphosphoinositide can be eluted by an ammonium acetate gradient in chloroform-methanol before monophosphoinositide, whereas the sodium salt is eluted long after the monophosphate. Such behavior amply illustrates the importance of considering the cationic composition of phospholipids. Plant phosphatidyl inositol was isolated by a similar technique (26).

Plant Sulfolipid



2,3-diacyl-1-(6-sulfo-α-D-quinovopyranosyl)-D-glycerol

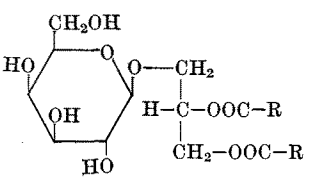
This lipid, the first known to contain a carbon-sulfur bond, was identified by Benson (62). He proposed the term "sulfolipid" for lipids containing a sulfonic acid group ($R–SO_3H$) to distinguish them from sulfatides which are esters of sulfuric acid ($R–O–SO_3H$). The structure first proposed by Benson was correct in most respects; but through elegant manipulations of small amts of material, he subsequently demonstrated the presence of 6-sulfo-6-deoxy-O-D-glucopyranose (6-sulfo-O-D-quinovopyranose) (63), a D-glycerol configuration (64), and two acyl groups (65).

Plant sulfolipid has been found in all photosynthetic plants in which it has been sought (63), including species of red, brown, green and blue-green algae, *Rhodospirillum rubrum,* barley, clover, spinach, chives, ryegrass, maize, and runner bean seedlings (66–68). Kates suggested the presence of a different sulfolipid in runner bean leaves (22), but this was not confirmed by subsequent investigations (66). Collier and Kennedy (67,68) reported three entities separated by paper chromatography which may involve sulfolipid bound to chlorophyll in certain plants, but only one in others.

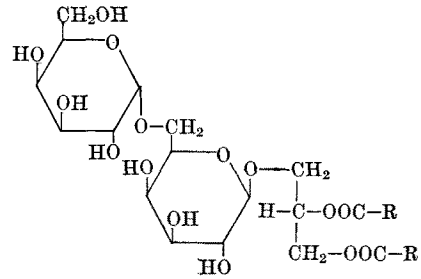
Plant sulfolipid has been prepared from crude lipid extracts on a modest preparative scale by three techniques. The phospholipids can be removed with an activated magnesium silicate column using the technique developed by Radin (69); sulfolipid can then be freed of other impurities by acetone precipitation (67,68), or with a diethylaminoethyl cellulose column (70). Or, starting with the total lipid extract, uncontaminated sulfolipid can be obtained by gradient elution from diethylaminoethyl cellulose columns (26).

Deacylated sulfolipid has been isolated as a crystalline cyclohexylamine salt after ion exchange chromatography (71).

Galactosyl Diglycerides



"Monogalactosyl Diglyceride"
2,3-Diacyl-1-(β-D-galactopyranosyl)-D-glycerol



"Digalactosyl Diglyceride"
2,3-diacyl-1-(α-D-galactopyranosyl-1,6-β-D-galactopyranosyl)-D-glycerol

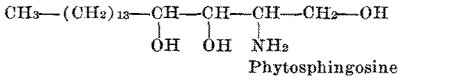
Monogalactosyl diglyceride and digalactosyl diglyceride were first identified by Carter et al. in benzene extracts of wheat flour (72). Partial separation of the two galactolipids was accomplished by countercurrent distribution and solvent fractionation. The structures were deduced (except for the configuration of the glycerol) from the chemical and physical properties of the deacylated materials. Later, "relatively homogeneous" materials were separated in Carter's laboratory by solvent fractionation and silicic acid chromatography, avoiding the tedious countercurrent distribution (73). Structure determination was completed with determination of the glycerol configuration by comparing the deacylated lipids with synthetic material (74). Miyano and Benson showed by quite different techniques that mono- and digalactosyl lipids of *Chlorella* had the same structures (64). Both galactolipids have been found in numerous plants and algae (26,29,31,70,73,75–79). The concn in chloroplasts is remarkably high; these lipids are probably universal constituents of photosynthetic tissue. The fatty acid component is predominantly linolenic acid in alfalfa (70), spinach (26), runner bean (78), and red clover (77). However, almost no linolenic acid is present in wheat flour galactosyl lipids (73), and none in *Anacystis nidulans,* a photosynthetic blue-green alga (80). Corn coleoptile lacks these lipids (81), but they are present in corn gluten (73).

Techniques of separating pure galactolipids from plant lipid extracts have been developed using multiple column techniques (26,70), or repeated silicic acid chromatography combined with ion exchange and solvent fractionation (78).

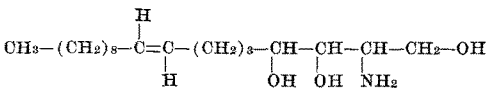
Plant Sphingolipids

The first reports of sphingolipids in higher plants were submitted almost simultaneously in 1953 (82,83), although there had been earlier indication of unidentified bases in plant lipid hydrolysates. References to this early work have been assembled by Carter et al. (82). Van Handel (83) isolated an acidic choline-free lipid from soybean phosphatides; this crystalline material with a P/N ratio of 2 and an equivalent weight of 1600 resisted mild alkaline hydrolysis as expected of a sphingolipid, but no further work has been reported on it. Carter et al. (82) separated "crude inositol lipid" from corn and soybean phosphatides by solvent fractionation; and from this isolated a mixed sphingolipid fraction by countercurrent distribution. Hydrolysis released not sphingosine, but other long chain bases. The predominant one from corn was named phytosphingosine and identified as D-*ribo*-1,3,4-trihydroxy-2-aminooctadecane (82,84). It is identical to a cerebrin base from mushrooms, yeast, and molds (85–87). Dehydrophytosphingosine, a *trans*-Δ^8 analog of phytosphingosine, is a minor base

in corn lipids, but the predominant one in flax and soybean (84). A 20-carbon analog of phytosphingosine is also present in soybean and peanut lipids (88), and in yeast (89).



Phytosphingosine

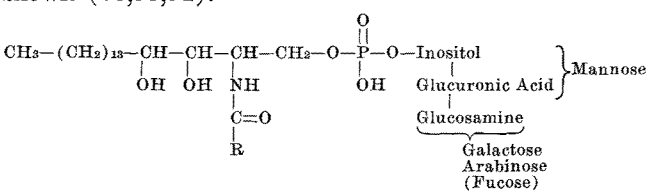


Dehydrophytosphingosine

Plant cerebrosides from wheat and runner bean leaves contain these same C_{18} bases, also dihydrosphingosine, an isomer of sphingosine, and unidentified bases (90–92).

Phytoglycolipid

The term "phytoglycolipid" is used by Carter (38) for a group of inositol sphingolipids obtained from a variety of oil seeds including soybean, corn, wheat, cottonseed, peanut, flax, and sunflower. It is soluble in pyridine, chloroform-methanol mixtures, and dimethyl sulfoxide, but insoluble in benzene, ether, and other nonpolar solvents. A partial structure for the phytosphingosine analog of phytoglycolipid is as shown (38,93,94).



The point of attachment and anomeric configuration of hexose moieties remains uncertain, but it is clear that the oligosaccharide(s) are derived from a common glucosamido-glucuronido-inositol unit, that the major portion of the mannose is attached to inositol or glucuronic acid, and that galactose, arabinose, and fucose are attached through glucosamine and are present in amts that vary somewhat with the plant source. The phytoglycolipids of corn and soybean contain no fucose; phytoglycolipid isolated from flax phosphatides (37) contained this sugar but appreciably less galactose.

The long-chain base composition of phytoglycolipids varies widely with the source (84).

	Dehydrophytosphingosine	Phytosphingosine
Flax	85%	15%
Soybean	80	20
Peanut	50	50
Corn	10	90

Fatty acids bound through the amide link are a mixture of unsubstituted and 2-hydroxy acids (38). In a convenient preparative method glycerolipids are removed from "crude inositol lipid" with milk alkaline hydrolysis, and phytoglycolipid is obtained as an amorphous white powder by precipitation of the alkali resistant sphingolipids from pyridine (38).

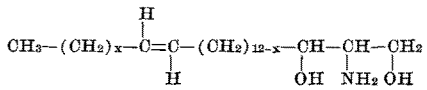
It is recognized that phytoglycolipid may be formed from a more complex lipid during mild alkaline hydrolysis used in its isolation (38). Countercurrent distribution of "crude inositol lipid" from flax in a butanol-water-methanol-hexane system separated a hexane-soluble material termed "phytoglycolipid precursor" (37). It contained appreciable amts of calcium and magnesium and may be a chelated double salt of phosphatidyl inositol and phytoglycolipid or a more complex parent. Similar Ca-Mg chelates of phosphatidyl inositol with phosphatidyl serine or phosphatidyl ethanolamine were found in the methanolic phase. These complexes are not broken by chromatography on paper or on silicic acid columns. The high calcium (0.65%) and magnesium (2.48%) and low monovalent cation content of "crude inositol lipid" is further indication of the strong binding of polyvalent metal ions by such phospholipids.

Another phytoglycolipid from flax may contain inositol, galactose, arabinose, and fucose (in the molar ratio 1 to 11–12 to 3 to 2) but no other sugars (37). The arabinose and fucose can be removed from the inositol-galactose oligosaccharide by mild acidic hydrolysis.

The function of phytoglycolipid is unknown. Analysis of corn seedlings for phytosphingosine at increasing times after pollination indicates that phytoglycolipid is probably not primarily a storage form (95).

Cerebrosides

The isolation of an impure glycoceramide fraction from wheat flour in Carter's laboratory (90,91) was the first conclusive proof of the occurrence of "cerebrosides" in plant lipids, although their presence had been suggested earlier (see reference in 91). The material isolated from wheat solvent fractionation and silicic acid chromatography was composed of glucose, α-hydroxystearic acid (the predominant acid), and four long-chain bases: phytosphingosine, dehydrophytosphingosine, dihydrosphingosine, and an isomer of sphingosine with the structure.



A glucocerebroside has also been isolated from runner bean leaves by Sastry and Kates (96). The α-hydroxy acids were mixed (C_{16} to C_{26}), as were the long-chain bases (C_{18} dihydrosphingosine was the most prevalent).

A less conventional "cerebroside" detected in wheat lipids is probably a trimannoside (73).

Proteolipid

If a proteolipid is defined as a class of protein-lipid molecules with solubility properties characteristic of a lipid rather than a protein, there is no doubt that such species exist in plant tissue. A particularly well-studied system of this sort is the isooctane soluble complexes between cytochrome c and phospholipids (97). In this case the stoichiometric complexes result from electrostatic attraction of the anionic lipids to the positively charged protein surface. Lipophilic species of this sort are unquestionably important in molecular organization of functioning biological systems, especially lipid-rich units such as mitochondria and chloroplasts. Polyvalent metal ions (such as calcium and magnesium) should be capable of firmly binding lipids through coordination complexes to other molecules including magnesium silicate (102), lipids (37), and polypeptides or amino acids. Cationically coordinated lipid-lipid systems may not be broken down during countercurrent distribution and subsequent chromatography (37). Certain proteolipids reported in plant lipid extracts may be of these types, but lipids with covalently bound peptides are also probably normal components of plants.

Kaufmann (98) has isolated a lipid fraction from chloroplast grana which contained alanine, serine, glutamic acid, and aspartic acid after countercurrent

distribution in a nonaqueous system. He speculated that the amino acids might be bound through acylphosphate bonds because (1) the amino acids were released more rapidly than ethanolamine in warm water, and (2) the amino groups of the bound amino acids were free to react with dinitrofluorobenzene. Benzinger et al. (99) found the same amino acids in proteolipid from bean leaf chloroplasts, sugar beet root leucoplasts, and wheat seeds. The nature of the lipid to peptide-nitrogen bond was not determined: the bond was not broken with boiling alcohol, but the amino acids were liberated by hydrolysis with 6N HCl and seemed to be firmly bound to one another, perhaps in a peptide bond. A list of references to other reports of proteolipids is included in Benzinger's article.

Zill and Harmon (32) extracted chloroplast lipids with boiling methanol, and separated a proteolipid fluff formed at the chloroform-water interface during a water wash. Folch has reported similar products in handling animal lipid extracts. The fluff was soluble in chloroform/methanol 2/1, but the protein was denatured upon flash evaporation of the solvent. Some of the lipid, including sulfolipid and galactolipid, could then be extracted with hexane, but some, unidentified, was more firmly bound. Carter and co-workers (73) fractionated benzene extracts of wheat flour lipids by a series of solvent fractionations and distributions. A high concn of peptide nitrogen was present in some fractions. "Bound fat" extracted from wheat gluten with boiling alcohol contains chloroform soluble lipids. Amino acid or protein in this material are not easily released (100). Zentner (101) has also isolated from wheat flour an ether and acetone soluble material containing protein or polypeptide.

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