Information concerning the kinds and composition of phospholipids in gymnosperm plants is negligible in the literature. Thus this study was undertaken to provide background knowledge for future comparative biochemical investigations. In this study, lipid was extracted by chloroform and methanol, and washed with distilled water to prevent the possible formation of phospholipid salts. Then, thin-layer, column and gas-liquid chromatographies were used for isolation of the phospholipids, separation of phospholipids into classes and analysis of fatty acids. Characterization of the different kinds of phospholipids was mainly by reagent spray and mild alkaline hydrolysis followed by identification with paper chromatography of the de-acylated phospholipids.

The total lipid content in endosperm and seedling of germinating Douglas fir seed was 40.5 and 17.5 percent (dry weight basis) respectively. The phospholipid contents were 3.6 and 22.6 percent of the total lipid respectively in the two kinds of tissues. The
composition of phospholipids in the two tissues varied. "Phosphatidic acid mixture" was the major component in both tissues representing 37.6 percent of lipid phosphorus in the endosperm and 30.1 percent in the seedling. Phosphatidyl choline comprised 30.6 percent of lipid phosphorus in the endosperm and 25.7 percent in the seedling, whereas phosphatidyl ethanolamine was 13.2 percent and 21.8 percent, phosphatidyl serine 14.7 percent and 10.3 percent, and amino acid containing phospholipids four percent and 12.2 percent respectively for these two tissues.
PHOSPHOLIPIDS IN GERMINATING
DOUGLAS FIR SEED

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PHOSPHOLIPIDS IN GERMINATING DOUGLAS FIR SEED

INTRODUCTION

Since the discovery of phosphorus-containing lipid by Fourcroy in 1793, a new area of lipid chemistry was instigated (21, p. 407). In addition to occurrence, most of the study on phospholipids has been concerned with chemical structure, methods for separation and characterization, and physiological roles in animal tissues. The early studies on plant lipid were concentrated on seed oils for their economic value, as the fatty acid contents and degree of unsaturation were important criteria for paint and oil industries in selecting raw materials. Owing to the increasing knowledge of lipid chemistry and function in animal tissues, the development of new techniques, such as gas-liquid, column, reverse-phase, paper and thin-layer chromatography and the availability of isotopic materials for metabolic study, the knowledge of lipid chemistry in higher plants has expanded very rapidly in the past fifteen years.

The functional roles of phospholipids in cellular and organellar membranes has been one of the centers of interest in recent years. In general, it is known that phospholipids, sterol esters and sphingomyelins are bound between two layers of protein to form membrane structures of cellular components (8, p. 1-16). Phospholipids could serve as ion-carriers to transport metal ions and
organic molecules through membranes (42, p. 61-85); they may
balance membrane charges thus maintaining ionic equilibrium and
facilitating selective permeability of the cell or organelle (31, p. 673-
679); they also may act as cofactors for some metabolic enzymes
(51, p. 391-401).

Seed is the main lipid-containing organ in plants, therefore, it
provides a ready source for lipid study. However, little is known
concerning the phospholipids of coniferous seeds, since the major
work reported in the literature has been on angiosperm seeds. As
Oregon is a major area for growing coniferous trees in the U. S.,
a large quantity of seed could be obtained easily. Thus, the isolation
and characterization of the phospholipids in Douglas fir seed was
chosen for this study. It is hoped that the information procured from
the study will provide useful background to understand the fat metab-
olism in gymnosperm seeds and to further explore the function of
phospholipids in cellular organelles. The results will also be useful
for comparative biochemistry.
REVIEW OF LITERATURE

Information regarding phospholipids in plants is reviewed in the following sections:

Naturally Occuring Phospholipids

Phosphoglycerides: The most abundant of naturally occurring complex lipids are phosphoglycerides, which in many cases comprise well over 70 percent of the total phospholipids (33, p. 42). Here, the term phosphoglycerides applies to serine, ethanolamine and choline phosphatidies. Structural characterization of these compounds by differential chemical hydrolysis had been handicapped by the migration of phosphate esters, but the final verification of the structures was made by hydrolysis with specific enzymes (33, p. 45-52; 3, p. 615-623). It was found that all naturally occurring phosphoglycerides have L-α-configuration (3, p. 615-623; 53, p. 612-617). The molecular architecture of these phosphoglycerides are:

\[
\begin{align*}
\text{L-α-phosphatidyl choline (PC)}
\end{align*}
\]
Detailed fatty acid compositions of different plant phosphoglycerides in various species are not well documented yet. However, generally the oleic/linoleic ratio is lower in phosphoglycerides than in glycerides in soybean and rape seeds was reported (40, p. 166-170). The same trend was also noticed by Ching (18, p. 722-728) in her Douglas fir seed germination study; in addition, a lower ratio of arachidic/linoleic in the phospholipids as compared to the glycerides was also observed. The following pathways of phosphoglycerides synthesis were proposed by Kennedy (50, p. 119-148):
Phosphatidic acid + RCOSCoA $\rightarrow$ D-1, 2 diglyceride

CDP-choline

Phosphatidyl choline

D-1, 2 diglyceride $\rightarrow$ S-adenosyl-methionine

CDP-ethanolamine

Phosphatidyl ethanolamine

Ca$^{2+}$

serine $\rightarrow$ CO$_2$

Phosphatidyl serine

Phosphoinositides: Because of the difficulties in isolation and purification, the chemical characterization of phosphoinositides has been revealed only in recent years. Klenk and Sakai (58, p. 254-255) in 1939 isolated inositol monophosphate among the hydrolysis products of soybean phosphatides. Woolley (81, p. 963-964) in 1942 isolated a rather pure inositide called lipositol from soybean. Later analysis showed that lipositol contained tartaric acid but not glycerol among its constituents. Another inositide was obtained by Malkin and Pool from peanut (55, p. 3470-3478) in 1953. A crystalline sample of phosphatidyl inositidic acid was isolated from wheat germ by Faure and Morelec-Coulon (23, p. 411-412). Also Folch (26, p. 252) succeeded in isolating a pure form of monoinositide from soybean and he found the molar ratio of primary amine:phosphoric acid: inositol: glycerol: monosaccharide:fatty acids to be 1:2:2:2:2:3. Recently, Wagenknecht and Carter (77, p. 2265-2268) obtained monophosphoinositide from peas in a Ca-Mg salt form. They also reported that
the peas had as high as five percent of monoinositide in its total lipids extract. During the study of inositol lipids, Carter and associates (14, p. 1309-1314) were able to isolate phospholipids containing inositol and a long chain base and they designated the lipids as phytoglycolipids. After the structural study by alkaline hydrolysis and chromatography, they proposed the compounds had the following structures:

\[
\begin{align*}
\text{CH}_3\text{(CH}_2\text{)}_3 & \text{-CH-CH-CH-CH}_2\text{-O-PO}_3\text{-inositol} \\
\text{OH} & \text{OH} \text{NH} \\
\text{COR} & \text{glucosamine} \\
& \text{hexuronic acid} \\
& \text{arabinose} \\
& \text{galactose} \\
& \text{mannose}
\end{align*}
\]

In general, all the naturally occurring phosphoinositides in plants are monophosphoinositides. Diphosphoinositides have been found only in animal brain tissues (27, p. 497-519). The chemical structure of mono- and diphosphoinositide are:

\[
\begin{align*}
\text{O} & \text{CH}_2\text{-O-C-R} \\
\text{R-C-O-CH} & \text{OH} \text{HO} \text{OH} \\
\text{CH}_2\text{-O-P-O-OH} & \text{OH} \text{HO} \text{OH}
\end{align*}
\]

Monophosphoinositide (PI)
Diphosphoinositide

There were two pathways proposed for the synthesis of phosphatidyl inositol (82, p. 225-264):

1. CDP-choline + L-phosphatidic acid $\rightarrow$ CDP-1, 2 diglyceride
   CDP-1, 2 diglyceride + inositol $\rightarrow$ Inositol phosphatide + CMP

2. Phosphatidic acid + CTP $\rightarrow$ CDP-diglyceride + P-P
   CDP-diglyceride + inostiol $\rightarrow$ Inositol phosphatide + CMP

*Phosphatidyl glycerol:* Older literature suggested the presence of polyglycerophosphate-containing lipids in plants. Recent radiochemical investigations conducted by Benson et al. (7, p. 189-195) showed compounds of this type which have the structures of phosphatidylyl glycerol (GPG) and diphosphatidyl glycerol. He found that in Scenedesmus, GPG constituted 41 percent of the total lipid P incorporated. In leaf tissues of higher plants, such as tobacco, sweet clover and barley had as much as 22 percent of total lipid P incorporated into GPG. Kates (46, p. 315-328) applied $^{32}$P on runner bean leaves and found the same 22 percent of total $^{32}$P absorbed in
GPG, while Wheeldon (79, p. 439-445) failed to find GPG in cabbage leaves by classical chemical analysis. The larger amount of GPG in plant tissues than animal tissues was thought to be related to the higher carbohydrate metabolic activity of plant tissues (52, p. 2293-2298). It has been proposed that the metabolic role of GPG may be to serve as phosphatidyl group precursor for other phosphatides is well as a large reservoir of readily available glycerophosphate for hexose synthesis (24, 185-192). The pathway of GPG synthesis was proposed by Kennedy and Weiss (49, p. 250-251) as:

"DPNH
 $\alpha$-GP-dehydrogenase"

\[ \text{Dihydroxyacetone phosphate} \rightarrow \text{D-glyceryl-1-phosphate} \]

\[ \text{D-glyceryl-1-phosphate} + \text{CTP} \rightarrow \text{CDP-glycerol} + \text{PP} \]

\[ \text{D-2,3-diglyceride} + \text{CDP-glycerol} \rightarrow \text{Phosphatidyl glycerol} \]

\[ \text{Phosphatidyl glycerol} + \text{CDP-diglyceride} \rightarrow \text{Diphosphatidyl glycerol} \]

\[ \rightarrow \text{(Cardiolipin in animal)} \]

The chemical structure of GPG is:

```
O
\[ CH_2-O-C-R \]
\[ R'-C-O-CH \]
\[ CH_2-O-P-O-CH_2 \]
\[ OH \]
\[ HC-OH \]
\[ CH_2OH \]
```
Phosphatidic acid: Chibnall and Chanon (17, p. 233-246) isolated a large amount of phosphatidic acid from cabbage leaves. Hanahan and Chaikoff (34, p. 191-198) suggested that phosphatidic acid was an artifact caused by hydrolysis of choline, serine and ethanolamine phosphatides by phospholipase during isolation. Benson and Mauro (7, 189-195) failed to find phosphatidic acid in a number of plants by $^{32}$P incorporation. Kates (46, p. 315-328), however, observed a high specific activity of phosphatidic acid after $^{32}$P treatment to the primary leaves of scarlet runner bean. The failure to find significant amount of phosphatidic acid and the observation of high specific activity of phosphatidic acid may indicate the high turnover rate of phosphatidic acid in plant tissues. The following synthetic pathway of phosphatidic acid was proposed by Bradbeer and Stumpf (12, p. 214-220) in peanut cotyledon:

$$\text{D-1,2-diglyceride} + \text{ATP} \rightarrow \text{Phosphatidic acid} + \text{ADP}$$

the chemical structure of phosphatidic acid (PA) is:

```
O
\begin{array}{c}
\text{CH}_2-O-C-R \\
\text{R'-C-O-CH} \\
\text{CH}_2-O-P-OH \\
\text{OH}
\end{array}
```
Lipid-amino acid complex: As reviewed by Zill (82, p. 225-264), the presence of lipid-amino acid complexes in plant tissues was reported by Kauffmann in spinach grana; Kauffmann and Kodding found that the amino acids are glycine, alanine, serine, glutamic acid and aspartic acid; Bezinger et al. again found the above five amino acids present in the phospholipids from chloroplasts of bean, sugar beet, sunflower and nettles, in the locusta of sugar beet and in wheat seed. Recent work of Brady (13, p. 105-119) on broad bean leaves indicated the incorporation of $^{14}$C-valine into lipids, and partial hydrolysis of the lipid amino acid complexes released free amino acids and peptides. The possibility that these lipid-amino acid complexes are involved in protein synthesis was mentioned by Hendler (39, p. 1466-1473). The possible chemical structure of lipid-amino acid complex (AP) is (44, p. 140-141):

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{CH}_2\text{-O-C-R} & \quad \text{R}\text{-C-O-CH} \\
\text{CH}_2\text{-O-P-O} & \quad \text{amino acids} \\
\text{OH} & \quad \text{or} \\
& \quad \text{peptide}
\end{align*}
\]
Distribution of Phospholipids

From the older published data, a considerable discrepancy was found in the contents of phospholipids in various seeds. Olcott and Mecham (64, p. 407-414) explained the variance by assuming that phospholipids in seeds are bound in a complex with protein and/or carbohydrate, from which they will be extracted in varying degrees by different solvent. In general, it appeared that in seeds phospholipids may comprise from 0.1 percent to as much as four percent of total lipids (58, p. 255), depending on species and the portion of seed examined. Soybean, cottonseed and peanut seeds were regarded as having a high phospholipids content of two percent on the average.

Kates (46, p. 315-328) examined the lipid compositions of runner bean leaves by combining the radiotracer techniques and paper chromatography, he found the distribution of phospholipids were lecithin 45 percent, phosphatidyl glycerol 22 percent, phosphatidyl ethanolamine 17 percent and phosphatidyl inositol nine percent.

Benson and Maruo (7, p. 189-195) used two-dimensional paper chromatography to separate the hydrolysis products of phospholipids from leaves of tobacco, sweet clover and barley. The percentage distribution of phosphorus, detected by counting the radioactivity of $^{32}$P after in vivo labelling, was lecithin 30-52.5 percent, phosphatidyl glycerol 22-24.9 percent, phosphatidyl inositol 14-22.4 percent,
phosphatidyl ethanolamine 1.4-8.6 percent and phosphatidyl serine 0.7-6.3 percent.

Wheeldon (79, p. 439-445) examined cabbage leaves by fractionating phospholipids through a silicic acid column and identified fractions by their hydrolysis products separated by paper chromatography. He found lecithin was 29 percent of phospholipids Pₚ, the highest, followed by phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl glycerol. The fatty acid composition of phospholipids was relatively simple and uniform. The main saturated fatty acid was palmitic acid while the main unsaturated fatty acids were oleic and linoleic acids. Lecithin contained almost exclusively unsaturated fatty acids.

Wagenknecht (77, p. 2265-2268) isolated a rather pure, white, fluffy powder of Ca-Mg salt of monophosphoinositide from lyophilized frozen peas. The yield of this salt amounted to about five percent of the total pea lipids.

While investigating the photosynthetic apparatus, Wintermans (80, p. 49-54) compared the lipid contents of leaves and chloroplasts and also compared yellow and green leaves. He found that spinach, beet, elder and bean seedling with green chloroplasts were particularly enriched in phosphatidyl glycerol and in mono- and digalactosyl lipids as compared to yellow leaves. Other phospholipids did not vary with leaf color and probably were not especially functional in
the photosynthesis. The composition of phospholipids are: PC* (38-56 percent), PE** (2-26 percent), PI*** (9-13 percent) and glycerol phosphate (0-3 percent).

Recently, the change of phospholipids and their fatty acid contents during different germination stages of Douglas fir seed was reported by Ching (18, p. 722-728). Total phospholipids increased from five percent of total fats of the non-germinated seed to 25 percent of the fully germinated seed. The fatty acid contents of phospholipids also increased from 1.9 mole percent to 14.7 mole percent of the total fatty acids in seed. The major components of fatty acids in phospholipids were linoleic (40-45 percent), oleic (13-26 percent) and palmitic (13-21 percent) acid. A sharp decrease of oleic acid (from 26 percent to 13 percent) and a slight increase of palmitic acid and linolenic acid during germination was noticed.

Isolation and Purification

Complex forms of lipid, protein and carbohydrate are commonly found in seeds, the lipid-protein complex in caster bean, lipid-carbohydrate complex in plants, and protein-lipid-carbohydrate complex in plants, and protein-lipid-carbohydrate complex in plants.
complex in wheat germ (54, p. 70-89) are some well-known examples. The X-ray diffraction study by Finean (25, p. 17-19) on animal cell membranes and the electron microscope study by Thomas et al. (73, p. 90-100) on chloroplasts of spinach, proved the existence of lipid complex in membranes. Chargaff and Cohen (16, p. 619-628) explained the conjugation as the valence bonds extended from the acidic lipid e.g. phosphatidyl serine or phosphatidyl ethanolamine to the basic protein and/or carbohydrate.

Because of the complex form of lipid in tissues, it is realized that complete extraction of lipids from tissues is almost impossible. Variation in extracting solvent, time and temperature would result in varying compositions of each lipid class. Hanahan (33, 1. 11-41) suggested that extraction at room temperature by chloroform-methanol mixture would be the most effective. Besides, phospholipids have a remarkable ability to solubilize many non-lipid components. In general, the substances encountered most frequently are sugars, free amino acids, sterols and sterol esters and many inorganic substances. For the purpose of removing these impurities, water and salt washing techniques were introduced by Folch (29, p. 833-841). Although salt washing will minimize the lipid loss during washing (28, p. 497-509), the possible complex formation between salt and lipid still remains as a problem. Hanahan (33, p. 11-41), therefore, suggested distilled water washing is the most
reliable method for purification.

Isolation of phospholipids in pure form has been a problem in lipid chemistry for years. During the past decade, new and improved procedures, especially chromatography, have been forthcoming and have allowed a better fractionation and enabled subsequent identification. These chromatographic methods are, column, thin-layer, gas-liquid and paper chromatography. Among them, the column chromatography gives more satisfactory results in fractionating large quantities of naturally occurring lipids into classes, which in turn, will provide enough quantity to allow complete identification. In general, there are three kinds of absorbents commonly used in column chromatography for phospholipid separation. They are aluminum oxide, silicic acid and diethylaminoethyl (DEAE) cellulose. Usually, aluminum oxide is good in its ability to separate choline-containing phospholipids from non-choline containing phospholipids (33, p. 11-41), but it was limited for a possible binding between the acidic phosphatides, such as serine and ethanolamine phosphatides and the basic absorbent. Silicic acid probably is the most widely used absorbent for phospholipid separation. It is good for separating the phospholipids into their main classes, also it is good for fractionation of neutral lipids. The shortcoming of silicic acid would be that it can not separate the phosphoglycerides from their corresponding lyso-compounds. Recently, DEAE cellulose was introduced
by Rouser et al. (67, p. 112-123). It is believed by the authors that this absorbent has the ability to separate phosphoglycerides from their corresponding lyso-compounds. Besides, it has the advantage of binding the oxidation products of phospholipids.

Characterization for phospholipids class can be done in many ways. Spraying with chemical reagent and calculating their $R_f$ values becomes the most convenient way for characterization. In addition, the partial hydrolysis method of Dawson (20, p. 45-53), the modified method of Benson and Maruo (7, p. 189-195) and the molar ratio determination after complete acid hydrolysis (33, p. 11-41) are the most popular and reliable ways for characterization. Information regarding recent progress in chromatography was reviewed by Marinetti (57, p. 1-20).

Functions

Early in 1935, Sinclair (68, p. 515-526) found that there was a difference in the incorporation of dietary fatty acids between tissues, he formulated the idea that there are two types of phospholipids, "metabolic phospholipids" and "structural phospholipids", in which the former has a rapid turn over rate when an uptake of fatty acids occurred, while the latter was only little affected. Even though this idea is not currently accepted, the postulation may have significance.
The main functions proposed for phospholipids in plants are:

**Constituents of cellular or organelle membranous structures:**

The concept of "bimolecule leaflet" by Davson and Danielli in 1952 was accepted as a model of cellular membranous structures (8, p. 1-16). Simply explained, this structure is formed as phospholipids sandwiched between two aqueous protein phases, with polar groups of lipid molecules sticking outward to form valence bonds with their adjacent protein. In general, mitochondria, chloroplasts and other isolated cell membranes consist of lipoprotein with 30-50 percent of lipids on a dry weight basis or over half lipids by volume (8, p. 1-16). An increase of root phospholipids during growth was observed by Hanson and his collaborators (38, p. 751-800). Membrane structures were expanded when the root cells developed from their meristematic tissues to maturity. There is almost twice as much phospholipid in the root mature cells as in meristematic zones (8, p. 1-16). Besides, it was reported that mitochondria membrane phospholipids increased with the development of micro pores in Tradescantia (4, p. 241-254).

**Function as "carrier" in transporting metal ions and organic molecules through membranes:** The chemical nature of phospholipids in membranes has been studied by Christenson and Hasting (19, p. 387-398). They found that the phospholipids have a cation-binding capacity with K or Na ions. Experimental evidence for their role as
metal ion "carrier" was afterward observed by Solomon (71, p. 57-110) in blood. He found the radioactive K and Na ions can be incorporated into phospholipids rapidly. In plants, the uptake rate of K ion in Zea mays root was studied by Hanson (38, p. 795-800). The results showed that the higher the phospholipid contents in the root section, such as expanding or maturation zone, the faster the rate in K ion uptake. In addition, Kahn and Hanson (45, p. 621-629) found the isolated corn root mitochondria were able to synthesize lecithin which appeared to facilitate oxidative phosphorylation and enhance the cell's ability to accumulate potassium. This is another example of ion uptake by membrane phospholipids, which in addition is associated with the respiratory enzyme systems.

Studies of the possibility of membrane phospholipid functioning in transporting organic molecules were mostly carried out with animal tissues (68, p. 515-526; 11, p. 120-139; 66, p. 169-182). The results showed that phospholipids act as an intermediate which is necessary for the re-synthesis of neutral lipids for cells after the digestion of triglycerides. Another possibility is that phospholipids may be involved in protein synthesis by transporting amino acids to the sites of protein synthesis (44, p. 140-141).

**Phospholipid effects on membrane permeability:** There is no direct evidence that phospholipid affects plant cell membrane permeability. However, when Gibson (31, p. 673-679) studied the fat
metabolism of rat liver cells it was suggested that the interconversion of phosphatidyl choline and phosphatidyl serine would result in balance of the membrane charge and influence the permeability of membranes. It is believed that such a mechanism could also occur in plant tissues. Recently, O'Brien (63, p. 1099-1107) examined very carefully the myelin membrane of nerves. He proposed that the saturation of fatty acids in phospholipids would also affect the membrane permeability; increasing the unsaturation of fatty acids leads to a more permeable structure.

**Phospholipids related to the enzyme systems:** Many investigations on enzyme systems in chloroplast are reported in the literature (24, p. 10-12; 78, p. 188-193). It is known that the enzymes in chloroplasts which are responsible for photosynthetic phosphorylation and the Hill reaction existed in a bound form as lipoprotein (60, p. 27-44). The lipid portion involved in the structure was examined by various investigators (9, p. 328-333; 10, p. 315-317; 80, p. 49-54) and found to be phospholipids, glycolipids and sulfolipids. It is believed that the phospholipids here are in the position to stabilize the enzyme systems of chloroplasts by the presence of hydrophilic groups in their structures (60, p. 27-44). However, the non-polar long chain fatty acid moiety was believed to contribute the hydrophobic binding of non-polar molecules, such as quinones and pigments which are important compounds participating in photosynthesis.
(60, p. 27-44). That phospholipids may play an important role in the electron transport system of mitochondria was demonstrated in animal tissues (1, p. 463-466; 22, p. 619-633). The results showed that phosphatidase may stop the electron transport chain and oxidative phosphorylation in mitochondria by destroying the integrity of lipo-protein. The lost ability can be restored by adding back phospholipids (32, p. 63-74). Also the role of phospholipids as a cofactor in muscle ATP-ase was found by Kielley and Meyerhof (51, p. 391-401).

The high phospholipid content of plant mitochondria was reported by Benson (9, p. 328-333). Although the exact role of phospholipids is not known yet, from the study of animal mitochondria, it is believed that the phospholipids may take part in the catalytic activity in the mitochondria enzyme systems (32, p. 63-74).

In summary, the importance of phospholipid in membrane structure is widely recognized but the chemical information and methods for examination are still far from satisfying.
MATERIALS AND METHODS

Materials

**Douglas fir seedling and endosperm:** Douglas fir seed was harvested near Corvallis in 1963 and stored at 4°C till used. Seed was first washed with dilute Arasan suspension (ethyl mercury phosphate, Du Pont Co.) to reduce fugal growth and stratified in petri-dishes at 4°C for three weeks, germinated at alternating daily temperatures of 30°C for 16 hours with 200 foot-candle florescent light and 20°C for eight hours in the dark. After one week's germination, seedlings with radicle length of 10-15mm were selected for this study. At this stage of germination, the dry weight of endosperm was about 2.6 times of the seedling. The selected material was then separated into seedling and endosperm with the help of forceps. The separated tissues were immediately dipped into a known quantity of methanol to stop enzymatic reactions. Moisture contents of both seedling and endosperm were determined on another sample of comparable material by drying at 85°C for 24 hours. The moisture contents were used for estimation of dry weight.

**Chemicals:** All the chemicals used in this study were reagent grade. Diethyl ether was distilled over sodium chips to remove peroxide. Chloroform, methanol, petroleum ether and ethanol were all re-distilled before use.
**Chromatographic materials:** Thin-layer chromatographic materials of silica gel G and silica Gel H were purchased from Brinkmann Instrument, Inc., Long Island, N. Y. Silicic acid used for column chromatography was from Nutritional Biochemicals Corporation, Cleveland, Ohio. Further purification was conducted by suspending the acid in water for 30 minutes, collecting the sediment, which has a particle size around 150-300 mesh and activating at 110°C overnight. For fatty acid analysis the liquid phase used in gas-liquid chromatography was diethylene glycol succinate purchased from Applied Science Laboratory, State College, Penn. and Wilken Instrument and Research, Walnut Creek, California. The inert support was 90-100 mesh chromosorb W, washed with acid and alcoholic base. The column packing material was prepared according to the procedure of Horning (43, p. 751-752). Aluminum tubing with 6 mm O. D. and 275-450 cm in length was used, filled column with about two grams of packing material per foot.

**Standard compounds:** Phosphatidyl inositol and phosphatidyl serine were from Sigma Chemical Company, St. Louis, Missouri, phosphatidyl choline from B. D. H. Laboratory Chemical Division, London, England. While phosphatidyl ethanolamine was from Dr. Rene Maier. Standard fatty acids or their esters were obtained from the Hormel Institute, University of Minnesota and the Applied Science Laboratory.
Lipid Extraction and Washing

The fresh seedling or endosperm was immersed in a tared bottle of methanol to prevent enzyme action. The tissue was weighed in the methanol to determine fresh weight. After adding a sufficient amount of chloroform, the material was then homogenized in chloroform (C) and methanol (M) (2:1 by volume) solvent mixture for ten minutes. The homogenate was filtered and filtrate was collected. Re-extraction of fats by the same solvent mixture was conducted twice on endosperm with time intervals of ten minutes and one hour, and three times on seedling with time intervals of ten minutes, one hour and two hours at room temperature with occasional stirring.

The volume of solvent mixture used for extraction generally was ten times the fresh weight of materials. The lipid extracts were combined and dried under reduced pressure (approximately 0.2 atmosphere) at 60°C overnight. The residue was designated as crude lipid.

The distilled water washing procedure by Folch (29, p. 833-841) was chosen for reduction of contaminants extracted by methanol. The procedure was dissolving crude lipid in 35 ml. of C:M (2:1 by vol.) solvent mixture, equilibrating the dissolved lipid twice with 52 ml. of distilled water in a separatory funnel, separating the water phase from the organic phase by centrifuging the mixture for 20
minutes at high centrifuge force, decanting water phase and drying the organic phase at 60°C under reduced pressure. This washed lipid was designated as total lipid (TL).

Completeness of extraction was checked by re-extracting the residue with 50-100 ml. of C:M (2:1 by vol.) and washed with distilled water, and checked the total phosphorus in the washed fats.

Chromatographic Methods for Phospholipids Fractionation

The polar lipids were separated from the extracted total fats by counter-current distribution in pre-equilibrated two phases of 87 percent ethanol and pet ether (30, p. 134-136). The ethanol phase which contained phospholipids and other neutral polar lipids was dried under reduced pressure and fractionated by silicic acid column into phospholipids and others according to the procedure of Hanahan (35, p. 54-65). Further fractionation of phospholipids into different classes was achieved mainly by thin-layer and column chromatography.

**Thin-layer chromatography:** Silica gel G neutral plate was used for qualitative separation and identification of phospholipids. The solvent systems of Skidmore (69, p. 471-475) and McKillican (59, p. 554-557) were used. Silica gel H alkaline plate was used for both qualitative and quantitative study of phospholipid classes. After developing the plate in solvent system of chloroform:methanol:
acetic acid:water (25:15:4:2) as described by Skipski (70, p. 374-378), the location of phospholipids was indicated by exposing the plate to iodine vapor. Each fraction was scraped from the plate by a razor blade, and eluted either by solvent mixture of C:M (2:1 by Vol.) or twice by 1 N methanolic HCl at 60°C for a half hour each. The first eluting method was used for characterization only, whereas the second eluting method was used for quantitative determination of phosphorus distribution.

Column chromatography: The column were wet packed by making a slurry of activated silicic acid in chloroform, followed by 100 ml. of methanol and 300 ml. of chloroform washing. The phospholipid dissolved in five ml. chloroform were charged on the column and eluted with different solvent systems. The eluate was collected by an automatic collector in test tubes, and the volume of eluate in each tube ranged from 4-7 ml. was measured. For the seedling, a loading rate of 36 μg P/gram of silicic acid was used with column size of three cm. x 15 cm. The elution pattern was chloroform (C): acetone (A) (1:1), A, C:M (9:1), C:M (6:1), C:M (4:1), C:M (1:1) C:M (1:2) and methanol with a flow rate of one ml./15 min. Total phosphorus content of each tube was determined by taking an aliquot of the eluate for localizing fractions of phospholipids. The method used for organic phosphorus determination was of Bartlett (6, p. 466-471). For the endosperm, a loading rate of 90 μg P/gram silicic
acid was applied on a column with the size of two cm. x 19.5 cm. and elution was conducted with flow rate of one ml./two minutes. The elution pattern was C:M (9:1), C:M (4:1), C:M (1:1) and methanol. Total phosphorus was determined in every other tube for localizing fractions of phospholipids.

Methods for Characterization of Phospholipids

Thin-layer chromatography was used as a general and rapid way for characterization. Both total phospholipids—and phospholipid fractions eluted from the column were checked for purity by one-dimensional or two-dimensional thin-layer chromatography, followed by identifying the spots in various ways: by iodine vapor for unsaturation (56, p. 708-727), ninhydrin for free amino groups (74, p. 627-699), Dragendorff spray for choline-containing compounds (76, p. 175-184), ammonium silver nitrate for inositol-containing compounds (65, p. 238-250), perchloric acid spray for phosphorus containing compounds (37, p. 1107-1112) and acid charring for detection of all the organic compounds present.

The mild alkaline hydrolysis method of Dawson (20, p. 45-53) was used to identify phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine. The hydrolysis was conducted at 37°C for 20 minutes. After the alkali was removed by ion-exchange resin (Amberlite-IRC 50 H⁺), the hydrolyzed mixture was partitioned
between water and chloroform-isobutanol. The deacylated phospholipids were in the water phase and were identified by paper chromatography. The organic phase contained mainly fatty acids, which were methylated with diazomethane in ether and analyzed by gas-liquid chromatography (18, p. 722-728).

Complete hydrolysis of phospholipids were conducted in vacuum sealed ampoules with 2 N HCl for 24 hours at 105-110°C. The molar ratio of glycerol (36, p. 813-828) to P (6, p. 466-471) of seedling phospholipids was estimated. For amino acid containing phospholipids, the water phase of hydrolysate was concentrated and amino acids were identified by paper chromatography.

**Method for Fatty Acids Analysis**

The procedure of Ching (18, p. 722-728) was used. Fatty acids were characterized by comparing their retention time with the known standards separated under the same condition in the same column. Relative quantities of fatty acids were obtained from counts of the disc integrator installed in the recorder. Distribution of counts was calculated as the weight distribution of various fatty acids.
RESULTS AND DISCUSSION

Lipid Content of Seed

The data summarized in Table I showed that endosperm was rich in lipid in comparison to the seedling tissue. The endosperm was 40.5 percent lipids of its dry weight, whereas the seedling was only 17.5 percent. Further examining the phospholipid contents of each, a reverse relationship was found; the seedling had a high phospholipid content of 22.6 percent (w/w) of its total lipids, while endosperm had only 3.6 percent. From the data obtained in related studies, it was found that the dry weight of endosperm in this stage was 2.6 times that of seedling on the individual basis. In order to correlate the lipid and phospholipid contents on the whole seed basis, the data for endosperm were recalculated and listed as $E^*$ in Table I.

From the correlated data, an average weight of 9.92 grams of total lipids should be extracted from the whole seed material, that would be 34 percent of total lipids on the dry weight basis. Of the total lipids, 85.7 percent would be distributed in endosperm, only 14.3 percent in the seedling. The phospholipid contents would be 6.2 percent of the total lipids, and the distribution of phospholipid was about 50 percent in endosperm and 50 percent in seedling. The abundant quantity of phospholipid in seedling is believed to be due to the synthesis of membrane structures during growth (8,p.1-16). Other
Table I. Contents of total lipids and phospholipids in endosperm (E) and seedling (S) of germinating Douglas fir seed

<table>
<thead>
<tr>
<th>Material</th>
<th>Fresh Weight (gram)</th>
<th>Dry Weight (gram)</th>
<th>Total lipids (gram)</th>
<th>phospholipids (gram)</th>
<th>% lipid Fresh wt.</th>
<th>% lipid Dry wt.</th>
<th>% phospholipid Fresh wt.</th>
<th>% phospholipid Dry wt.</th>
<th>Total lipid wt. basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>27.5</td>
<td>12.3</td>
<td>5.11</td>
<td>0.177</td>
<td>18.6</td>
<td>41.5</td>
<td>0.64</td>
<td>1.43</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>29.5</td>
<td>13.2</td>
<td>5.19</td>
<td>0.188</td>
<td>17.5</td>
<td>39.4</td>
<td>0.64</td>
<td>1.42</td>
<td>3.6</td>
</tr>
<tr>
<td>Av.</td>
<td>28.5</td>
<td>12.8</td>
<td>5.15</td>
<td>0.183</td>
<td>18.1</td>
<td>40.5</td>
<td>0.64</td>
<td>1.42</td>
<td>3.6</td>
</tr>
<tr>
<td>S</td>
<td>36.4</td>
<td>5.8</td>
<td>1.00</td>
<td>0.222</td>
<td>2.74</td>
<td>17.3</td>
<td>0.61</td>
<td>3.83</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>65.9</td>
<td>10.4</td>
<td>1.84</td>
<td>0.414</td>
<td>2.80</td>
<td>17.7</td>
<td>0.63</td>
<td>3.98</td>
<td>23.0</td>
</tr>
<tr>
<td>Av.</td>
<td>51.2</td>
<td>8.1</td>
<td>1.42</td>
<td>0.318</td>
<td>2.77</td>
<td>17.5</td>
<td>0.62</td>
<td>3.91</td>
<td>22.6</td>
</tr>
<tr>
<td>E*</td>
<td>47.0</td>
<td>21.1</td>
<td>8.50</td>
<td>0.302</td>
<td>18.1</td>
<td>40.5</td>
<td>0.64</td>
<td>1.42</td>
<td>3.6</td>
</tr>
<tr>
<td>E*+S</td>
<td>98.2</td>
<td>29.2</td>
<td>9.92</td>
<td>0.620</td>
<td>10.1</td>
<td>34.0</td>
<td>0.63</td>
<td>2.12</td>
<td>6.2</td>
</tr>
</tbody>
</table>

* E: endosperm recalculated to whole seed basis.

°: phospholipid was obtained by charging total lipids on silicic acid column eluting neutral lipids into fractions by the solvent system of Barron and Hanahan (5, p. 493-503).
seeds with high lipid contents such as soybean, cottonseed and peanut also have high phospholipid contents, ranged from two to 3.5 percent of their total lipids (58, p. 255). The material used is germinated and a trend to increased phospholipid during germination had been reported (18, p. 722-728). This could be the reason that accounts for the higher contents of 6.2 percent comparing with two - 3.5 percent in other fat-rich ungerminated seeds.

From the same table it is interesting to find that the percent of phospholipids based on fresh weight was almost identical in both kind of tissues. This might indicate a constant relationship of phospholipids to the degree of hydration of the tissue.

The methods used for separating phospholipid from the total lipid extract gave similar results, that was 22.2 and 23 percent of phospholipid on the total lipid weight basis. These methods are counter-current distribution followed by column chromatography and direct separation of phospholipids by eluting total lipids with step-wise solvent systems (5, p. 493-503).

Composition of Phospholipids

From thin-layer and column chromatography, there were five main components in both endosperm and seedling phospholipids (see Fig. 1, 2 and Table II, III), they were "phosphatidic acid mixture" (fraction one), phosphatidyl ethanolamine (fraction two),
Material charged: 100.6 mg (2300 ug P)
Wt. of silicic acid: 26 grams
Column size: 2 cm x 19.5 cm
Collection time: 10 min./tube

Figure 1. Elution pattern of endosperm phospholipids by silicic acid column chromatography
Material charged: 0.1185 gram (2130 ug P)
Wt. of silicic acid: 60 grams
Column size: 3 cm x 15 cm
Collection time: 60 min./tube

Figure 2. Elution pattern of seedling phospholipids by silicic acid column chromatography
Table II: Characteristics of phospholipids isolated from seedling material separated by thin-layer chromatography

<table>
<thead>
<tr>
<th>Fractions</th>
<th>%P</th>
<th>Glycerol/P</th>
<th>I₂</th>
<th>Ninhydrin</th>
<th>Dragendorff</th>
<th>AgNO₃</th>
<th>NH₄OH</th>
<th>Ammonium Molybdate Perchloric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>I &quot;phosphatidic acid!&quot;</td>
<td>30.1</td>
<td>0.78</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II phosphatidyl ethanolamine</td>
<td>21.8</td>
<td>1.45</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>0.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III phosphatidyl serine</td>
<td>10.3</td>
<td>0.78</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV phosphatidyl choline</td>
<td>25.7</td>
<td>0.99</td>
<td>---</td>
<td></td>
<td>++</td>
<td>-</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V amino acid containing phospholipids</td>
<td>12.2</td>
<td>3.37</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table III. Characteristics of phospholipids isolated from endosperm material separated by thin-layer chromatography

| Fractions                     | %P  | I<sub>2</sub> | Ninhydrin | Dragendorff | AgNO<sub>3</sub> | NH<sub>4</sub>OH | Perchloric Molydate | Ammonium Molydate | Perchloric acid |
|-------------------------------|-----|---------------|-----------|-------------|------------------|----------------|---------------------|--------------------------------|
| I "phosphatidic acid"        | 37.6| +             | -         | -           | -                | -              | +                   | +                                      |
| II phosphatidyl ethanolamine | 13.2| +             | +         | -           | -                | -              | +                   | +                                      |
| III phosphatidyl serine       | 14.7| +             | +         | -           | -                | -              | +                   | +                                      |
| IV phosphatidyl choline       | 30.6| +             | -         | +           | -                | -              | +                   | +                                      |
| V amino acid containing phospholipids | 4.0 | +             | +         | -           | -                | -              | +                   | +                                      |
phosphatidyl serine (fraction three), phosphatidyl choline (fraction four) and amino acid containing phospholipids (fraction five). Quantitative data from TLC* shown: "phosphatidic acid mixture" had the highest concentration of 37.6 percent total lipid P in the endosperm and 30.1 percent in the seedling. Phosphatidyl choline was the second major component comprising 30.6 percent total lipid P in the endosperm and 25.7 percent in the seedling. Other components varied in their concentration as: in the seedling, phosphatidyl ethanolamine 21.8 percent, amino acid containing phospholipids 12.2 percent and phosphatidyl serine 10.3 percent; in the endosperm, phosphatidyl ethanolamine 13.2 percent, phosphatidyl serine 14.7 percent and amino acid containing phospholipids four percent. The first fraction was designated as "phosphatidic acid mixture", because of its $R_f$ value on two-dimensional TLC compared to phosphatidic acid in the solvent systems of chloroform:methanol:ammonium hydroxide (65:35:2), chloroform:methanol:ammonium hydroxide (35:65:2) and chloroform:methanol:water (65:25:1), diisobutyl ketone:acetic acid:water (40:25:5). Furthermore, the molar ratio of glycerol/P is approximately one (Table II) which indicated phosphatidic acid as well. Further separating this fraction by TLC with the solvent

*TLC: Thin-layer chromatography
systems of chloroform:methanol:water (65:25:1) and diisobutyl ketone:acetic acid:water (40:25:5) (59, p. 554-557), it appeared that in addition to phosphatidic acid there was a sterol-containing compound which showed red color during acid charring, and possibly two glycolipids, and one unknown, as estimated from their $R_f$ values. Similar results have recently been reported by McKillican (59, p. 554-557). She found that in wheat endosperm, the phospholipid fraction was accompanied by one sterol-containing lipid and several glycolipids and unknowns, although information about their detailed chemical characteristics was not mentioned in that paper.

Isolation of large quantities of phosphatidic acid from cabbage leaves was first reported by Chibnall and Channon (17, p. 233-246). It was also found that wheat germ had at least 42 percent of total lipid $P$ was in this form (15, p. 853-864). Recently, Nielson (62, p. 173-174) reported a high amount of phosphatidic acid in soybean as well. However, some investigations (34, p. 191-198; 47, p. 575-589) suggested that phosphatidic acid is an artifact caused by enzymatic hydrolysis of other phosphatides during isolation. In this study, methanol will stop all the enzymatic reactions during separation of the tissues, therefore, the phosphatidic acid probably is not an artifact.

The next major component in the material studied was phosphatidyl choline, ranged from 25.7 to 30.6 percent of the total lipid
phosphorus. In fact, phosphatidyl choline was reported as highest in quantity in phosphorus-containing compounds extracted from other plant materials.

It is rather surprising that in this study there was no phosphatidyl inositol detected. In general, phosphatidyl inositol has been regarded as a widespread phosphatides in seeds (72, p. 895-905). However, Wheeldon (79, p. 439-445) also failed to detect the presence of phosphatidyl inositol in cabbage leaves.

Fractions two and three were further identified by Dawson's mild alkaline hydrolysis (see Fig. 3) to be phosphatidyl ethanolamine and phosphatidyl serine. Comparing the relative amount of phosphatidyl ethanolamine and phosphatidyl serine present in seedling and endosperm, Table IV shows that seedlings had twice as much PE as PS, whereas endosperm had about the same quantity of PE and PS. A greater abundance of PE than PS in plant leaf tissues was reported by Benson and Kates (7, p. 189-195; 46, p. 315-328).

From the same table, it is obvious that seedlings had 12.2 percent of total lipid P in the form of amino acid containing phospholipids, which is about three times that of endosperm material in their relative quantity. The higher proportion of amino acid containing phospholipids in broad bean leaves was recently reported by Brady (13, p. 105-119). He found that the lipid bound amino acids could reach one percent of total lipids. If broad bean leaves also had 20 percent
Figure 3. Paper chromatogram of de-acylated phospholipids from seedling
Table IV. Comparison of recovery and percentage distribution of P in seedling and endosperm phospholipid fractions separated by thin-layer chromatography (TLC) and silicic acid column chromatography (SACC)

<table>
<thead>
<tr>
<th>Materials</th>
<th>Endosperm</th>
<th>Seedling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TLC</td>
<td>SACC</td>
</tr>
<tr>
<td><strong>P Charged (µg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td><strong>% P recovery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td><strong>I phosphatidic acid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>37.6</td>
<td></td>
</tr>
<tr>
<td><strong>II phosphatidyl ethanolamine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>12.4</td>
<td></td>
</tr>
<tr>
<td><strong>III phosphatidyl serine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>15.3</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td><strong>IV phosphatidyl choline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>29.2</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>31.9</td>
<td></td>
</tr>
<tr>
<td><strong>V amino acid containing phospholipids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>4.1</td>
<td></td>
</tr>
</tbody>
</table>
of phospholipids in their total lipids, then the amino acid containing phospholipids would reach five percent of total phospholipids. In the germinated seed, seedling is the growing part and is responsible for all the synthetic processes, therefore, more of phospholipids involved in binding amino acids for their transporting might be expected. After complete acid hydrolysis in sealed tubes, amino acid containing phospholipids in seedling released five amino acids: alanine, serine, glutamic acid, tryptophan or methionine and one unknown, as shown in Figure 4. Whether these amino acids are esterified singly or in a small peptide chain is not discernable. By the ratio of glycerol/P in Table II, one could speculate that this fraction is a complex one. Further work will be needed to characterize its chemical nature.

The variation in the ratio of glycerol/P in Table II could be attributed to (1) incomplete hydrolysis, since glycerol was determined in the aqueous phase of the hydrolysate while phosphorus was determined on the total material; (2) uncontrollable factors, such as absolute acidity for color development and degree of digestion during phosphorus determination.

**Fatty Acid Composition**

Fatty acid analysis on PE, PC and PS was shown on Table V. Apparently, palmitic (22-53.6 percent) and linoleic (36.8-47.7 percent) were the two major fatty acids in the phosphoglycerides which
Figure 4. Paper chromatogram of amino acids from acid hydrosate of seedling amino acid containing phospholipids
Table V. Fatty acid composition in different phospholipids of seedling

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Myristic (C(_{14:0}))</th>
<th>Palmitic (C(_{16:0}))</th>
<th>Palmitoleic (C(_{16:1}))</th>
<th>Stearic (C(_{18:0}))</th>
<th>Oleic (C(_{18:1}))</th>
<th>Linoleic (C(_{18:2}))</th>
<th>Linolenic (C(_{18:3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>II phosphatidyl ethanolamine</td>
<td>2.91</td>
<td>26.1</td>
<td>8.06</td>
<td>3.77</td>
<td>7.55</td>
<td>42.5</td>
<td>9.15</td>
</tr>
<tr>
<td>III phosphatidyl serine</td>
<td>--</td>
<td>53.6</td>
<td>9.42</td>
<td>--</td>
<td>--</td>
<td>36.8</td>
<td>--</td>
</tr>
<tr>
<td>IV phosphatidyl choline</td>
<td>--</td>
<td>22.0</td>
<td>6.50</td>
<td>9.00</td>
<td>14.8</td>
<td>47.7</td>
<td>--</td>
</tr>
</tbody>
</table>
account for almost 80 percent of total fatty acids in phosphoglycerides. Other fatty acids were palmitoleic (6.5-9.4 percent), oleic (7.5-14.8 percent), stearic (3.9-9.0 percent) and linolenic (0-9.2 percent) acid. The same table also shows that the kind of fatty acids and their quantity present varied with different classes of phospholipids. This difference was noticed in animal tissues (58, p. 231-264), but no specific data are available in plants which could be used for comparison. The high contents of palmitic and linoleic in soybean and rape seed was reported by Hilditch and Pedelty (41, p. 1964-1972). Recently, the analysis of fatty acids in the phospholipid of germinating Douglas fir seed (18, p. 722-728) also showed the major fatty acids to be linoleic (40.3 percent), palmitic (15.4 percent) and oleic (22.4 percent) acid. The failure to show high contents of oleic acid in this analysis possibly would be clarified by analysis of fatty acid contents of the other two fractions. In the present study, the quantity obtained for amino acid containing phospholipids was too small to provide an accurate analysis, while the mixture fraction one needs further fractionation before fatty acid analysis could be conducted profitably.
Comparison of Method for Separation

The result of silicic acid column chromatography (SACC) and thin-layer chromatography (TLC) for separation of phospholipids were compared in Table IV. For endosperm material there is a very close relation between SACC and TLC separation. In fact, SACC can not separate the amino acid containing phospholipids from phosphatidyl choline by the eluting solvent used, therefore, the data shown on column chromatography always have fraction four and five together. The discrepancy between SACC and TLC in seedling phospholipids separation could result from the different solvent used for elution. Using chloroform:acetone (1:1) and acetone for glycolipids elution (75, p. 3-6) also possibly brought down some phospholipids which had short chain fatty acid moieties. The TLC used to detect purity of phospholipid fractions separated by column also shown overlapping of fractions. This may possibly provide another reason for the discrepancy in phosphorus distribution.

A very low recovery of phosphorus in the column chromatographic results of endosperm phospholipids might be attributed to the inaccurate procedure for P determination. The column chromatography of these phospholipids should be repeated for reliable data. Nevertheless, TLC results were consistent and the method would be of value for quantitative analysis of phospholipids in small quantities of several milligram range.
SUMMARY

1. Endosperm of germinating Douglas fir seed is rich in lipids comprising 85 percent of the extracted total lipids from seed, while seedling tissue has only 15 percent. The weight of phospholipids, however, distribute equally in both kinds of tissues.

2. The kinds of phospholipids in both endosperm and seedling found to be comparable by thin-layer chromatography and silicic acid column chromatography separation. They are phosphatidic acid mixture, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine and amino acid containing phospholipid. Among them, phosphatidic acid mixture and phosphatidyl choline are the two major components comprising 55.8 percent of seedling lipid P and 68.2 percent of endosperm lipid P.

3. Fatty acid composition of seedling serine, ethanolamine and choline phosphatides shows that palmitic and linoleic acid are the two major components, composed from 68.6 to 90.4 percent of the total.

4. Thin-layer chromatography for separation of phospholipid is more applicable than the silicic acid column chromatography as a high percentage recovery of lipid P could be obtained on small quantity of original mixture with good separation within a short time. However, silicic acid column chromatography with modification of eluting system used might be desirable for separation of
larger quantity of phospholipids into fractions which could provide enough materials for complete characterization.
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