Phospholipids in the Uredospores of *Uromyces phaseoli*

I. IDENTIFICATION AND LOCALIZATION

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ABSTRACT

Utilizing paper, thin layer and gas chromatography, the phospholipids of dormant and germinating spores have been isolated and identified. The identifications were based upon agreements of retardation factor values between the unknowns and reference compounds and their derivatives. Quantitative analysis of components, color reactions and specific labeling experiments were also used to support certain identifications. At least three, and usually more, criteria were used for each phospholipid that was definitively identified.

The major phospholipids of *Uromyces phaseoli* are phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, or cardiolipin, phosphatidylinositol, and another phosphoinositol were present as minor components. In germinating spores, phosphatidylmonomethylethanolamine, phosphatidyltrimethylethanolamine, phosphatidylserine, and phosphatic acid were detected in trace amounts. The presence of these common intermediates in the biosynthesis of phospholipids indicates that phospholipid synthesis is active during the germination process. Except for the absence of phosphatidylglycerol, the types of phospholipids present are similar to the host plant. Germ tube wall preparations were found to contain phosphatidylethanolamine and phosphatidylethanolamine in about the same proportion as that observed in resting spores, while the proportion of diphasphatidylglycerol was about three times higher. An unidentified phosphorous-containing lipid was also a significant component of the total phospholipids extracted from germ tube walls.

It has long been held that neutral lipids may play a significant role during germination of uredospores of rust fungi (1, 8). In the later stages of germination, it is clear that they may serve as a source of energy. In the initial stages of germination, endogenous carbohydrates appear to be the chief substrates utilized, and the total amount of lipid is not changed extensively (8). However, certain fatty acids, such as cis-9,10-epoxyoctadecanoic acid, are rapidly metabolized during the first few hours. Spores also contain a highly active lipase whose temperature optimum coincides with the optimum temperature for the initiation of the germination process (16). It is possible that the lipase functions to provide certain fatty acids which are utilized in the synthesis of other lipids such as phospholipids.

During the germination process, the uredospores are in a highly active state. There is a synthesis of new germ tube wall, and as this structure is formed, new membranes containing phospholipids are required. Ultrastructural studies with *Puccinia graminis tritici* have revealed that extensive changes in the endoplasmic reticulum and lipid droplets occur during germination (28, 32). Thus it would appear that a significant turnover or relocation of phospholipids occurs during the germination process.

There is relatively little known about phospholipids in spores, especially during germination, and the identity of phospholipids in obligately parasitic spores has not been previously reported. The first paper of this series deals with the methods used in these studies, the identification of phospholipids in resting and germinating spores of *Uromyces phaseoli*, and the identification of phospholipids in the germ tube wall. The second paper (18) is concerned with the metabolism of phospholipids, especially biosynthetic pathways.

MATERIALS AND METHODS

Uredospore Production. Bean rust uredospores, *Uromyces phaseoli* (Pers.) Wint. var. *typica* Arth., were cultivated as described by Trocha and Daly (30). Phosphorous-32-labeled uredospores were obtained by the same method except a dilute H$_3$PO$_4$ solution (approximate pH of 5.5) was applied to the soil in which infected bean plants were growing. A total of 5 mc of radioactivity was applied to the soil of 12 pots over a 4-day interval, beginning the day flecking was first visible. The specific radioactivity of different spore collections ranged from 4,000 cpm/mg to 7,000 cpm/mg as determined by liquid scintillation counting.

Germination of Spores. The germination procedures are given in an accompanying paper (18), and the isolation of the germ tube wall will be described in detail elsewhere.

Extraction of Phospholipids. All phospholipid extractions were made with a chloroform-methanol (2:1, v/v) mixture. The spores were broken with a high speed oscillating shaker using a 10-ml stainless steel container and steel balls (¼ inch diameter). Up to 1 g of spores (if germinated spores were being broken the glass plug used to collect the spores was also inserted), 4 mg of steel balls, and 5 to 6 ml of the chloroform-methanol mixture were placed in each stainless steel container. The container was then oscillated three times for 3-min periods, allowing 5 min between each oscillation period. Spore breakage was performed at 4 C. Essentially 100% breakage occurred when the spores were treated in this manner. The contents of

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the container were then quantitatively transferred to a 40-ml centrifuge tube and extracted three times with 10 ml of the chloroform-methanol mixture. The extracts were combined, and the solvent was evaporated at 40 C under a stream of nitrogen. A minimal volume of the chloroform-methanol mixture was then used to redissolve the oily residue prior to application to thin layer chromatography plates. The washing procedure described by Folch et al. (11) was not used with the chloroform-methanol extract of endogenous 32P-labeled uredo-sores, because it was found that some of the polar phospholipids were lost in the saline solution. However, when spores germinated on 32P-labeled substrates were extracted with the chloroform-methanol mixture, the 0.29% NaCl wash was employed, since the chloroform-methanol extract contained an appreciable quantity of 32Cl-labeled, nonphosphorus-containing components which were removed by the saline wash.

Thin Layer Chromatography and Paper Chromatography. The preparation of the Silica Gel G plates has been previously described (17). Plates were air dried for 2 hr and either used in this form or activated at 100 C for 1 hr prior to use. For the preparation of five Silica Gel H plates, 45 ml of 0.1 M Na2CO3 was mixed with 30 g of Silica Gel H (Brinnman). The plates were air dried for 2 hr and then activated at 100 C for 1 hr. The above procedures were employed for the preparation of plates with a 0.25 mm thickness. When preparative thin layer chromatography plates (0.75 mm thickness) were required, the quantities of silica gel and water were increased by a factor of three. The following solvent systems were utilized for thin layer chromatography: A: chloroform-methanol-water (66:30:4, v/v/v) (25); B: chloroform-methanol-6 N ammonium hydroxide (30:65:5, v/v/v) (26); C: chloroform-methanol-pyridine-2 N ammonium hydroxide (35:12:65:1, v/v/v/v) (27); D: chloroform- methanol-7.4 N ammonium hydroxide (66:17.3, v/v/v) (31); E: methanol-6 N ammonium hydroxide-water (60:10:30, v/v/v/v) (26). Solvent systems B and C were used in conjunction with unactivated Silica Gel G plates and the Silica Gel H plates, respectively.

Solvent systems for ascending one-dimensional paper chromatography on Whatman No. 1 paper were: F: phenol-water (100:38, w/v) (3); G: 1-butanol-propionic acid-water (142:71:100, v/v/v) (2); H: 1-butanol-glacial acetic acid-water (77:6.17:100, v/v/v) (19); J: isopropanol-7.4 N ammonium hydroxide-water (7:1.2, v/v/v) (24). Solvent system K was 1-butanol-phenol-80% formic acid-water (50:50:3.5, v/v/v) saturated with KCl (5). Whatman No. 4 paper, which had been washed with 1 N KCl and dried, was used with solvent system K.

Detection of Components on Chromatograms. Detection of amino groups on both paper and thin layer chromatograms was accomplished by spraying the chromatogram with a ninhydrin solution. Phosphorous-containing components were detected on thin layer chromatography plates by the method of Dittmer and Lester (10). Detection of phosphorous-containing samples on paper chromatograms was carried out according to Dawson et al. (9). After spraying, exposure of the paper chromatogram to ultraviolet light caused the appearance of the spots in about 2 hr. An iodine spray, as described by Brante (4), was used for detection of monomethyllethanolamine, dimethyllethanolamine, and choline on both thin layer and paper chromatograms. In addition, the procedure described by Marinetti (22) was found to be very reliable for the location of choline on paper chromatograms.

When radioactive samples, either 32P or 32C, were involved, autoradiograms were prepared by exposure of the thin layer plate or paper chromatogram to No-screen x-ray film (Eastman Kodak Co., Rochester, N. Y.). For recovery of samples after thin layer chromatography, the bands were located either by autoradiography or by spraying the margin of the plate with the appropriate reagent. The adsorbent of the desired area from the unsprayed region of the plate was removed and extracted three times. Usually the silica gel was extracted with the chloroform-methanol mixture; however, for the inositides, chloroform-methanol-2 N ammonium hydroxide (35:25:20, v/v/v) was used.

Hydrolysis Procedures. Decaylation of the phospholipids was carried out according to the modified method of Card et al. (6).

Acid hydrolysis of phosphatidylethanolamine was accomplished with 0.2 N methanolic HCl in a sealed test tube at 60 C for 4 hr (6). Phosphatidylmonomethylethanolamine, phosphatidyltrimethylethanolamine, and phosphatidylcholine were hydrolyzed by the procedure of Goldfine (12). The acid was removed from the samples at 40 C under a stream of nitrogen with intermittent washing of the walls of the test tube with small volumes of methanol. The products were then dissolved in distilled water before being applied to chromatograms.

Two hydrolysis procedures were used for the phospholipids thought to contain inositol. When the inositol was qualitatively determined by gas chromatography, the phosphatidylinositol was hydrolyzed in a sealed test tube at 100 C for 5 hr in 1.0 ml of 1 N sulfuric acid. These conditions were necessary for the complete hydrolysis of the inositol phosphate that was initially formed. The acid was neutralized with Dowex I-X8 (carbonate form, Baker, Chemical Co.) (20). One milliliter of distilled water was then added, and the contents were shaken vigorously. After centrifugation the clear supernatant containing the myoinositol was removed. This extraction procedure was repeated five times, and the combined supernatants were taken to dryness under a stream of nitrogen.

The second hydrolysis procedure was used when determination of the inositol to phosphate ratio was required. The inositol was placed in a thick walled test tube and 1.0 ml of 5.6 N HCl was added. The sample was frozen, thawed under vacuum, rerozen, and sealed under vacuum. Hydrolysis was carried out for 48 hr at 120 C (13). After opening the tubes, the HCl was removed under vacuum over KOH pellets. One milliliter of distilled water was added to the dry sample. The lipid material was extracted by adding 1.0 ml of diethyl ether, shaking the test tube vigorously, and removing the upper layer after centrifugation. The ether extraction was repeated two times, and the ether layer was not retained for further analysis. The aqueous sample was taken to dryness under a stream of nitrogen, and a known volume of distilled water was added.

Quantitative Analysis of Inositol and Phosphorous. An aliquot of the hydrolyzed phosphatidylinositol was diluted to 2 ml with a sulfuric acid solution so that the final sulfuric acid concentration was 0.4 N. The phosphorous was then determined by the method of Chen et al. (7), except that the volumes of the reagents were reduced by 50% to increase the sensitivity of the assay. In all assays, standard curves were prepared when an unknown sample was analyzed. For the quantitative analysis of inositol, the colorimetric method described by Lornitzo (21) was used. A microcell with a 1-cm light path was employed, and standard curves were prepared each time an unknown sample was analyzed.

Gas Chromatography. Gas chromatography of myoinositol was carried out with a F and M Model 5750 Research Chromatograph equipped with a hydrogen flame detector. The silylation of myoinositol was accomplished by dissolving the sample in dry pyridine and adding trimethylchlorosilane (Pierce Chemical Co.) and hexamethyldisilazane (Pierce Chemical Co.). The respective concentrations were 7:1:2 (v/v/v). After
vigorously shaking, the mixture was allowed to stand at room temperature for 20 min with intermittent shaking, after which it was injected into the gas chromatograph. Two columns were used to detect the presence of myoinositol. A 9 ft x 1/4 inch stainless steel column, packed with 3% SE-52 (Analabs, Hamilton, Conn.) on Anakrom SD, 99% mesh (Analabs), was prepared by the procedure of Swelley et al. (29). The chromatography was temperature programmed to hold at 120 C for 6 min, then to increase 6 C/min to 185 C, and to hold at 185 C. Mannose exhibited a retention time of approximately 28 min. Mannose, which had a retention time of approximately 28 min, was used as an internal standard. A relative retention time of myoinositol was calculated by dividing the retention time of myoinositol by the retention time of mannose. The second column was a 2 ft x 1/4 inch stainless steel column packed with 3% SE-52 (Analabs) on Anakrom ABS, 99% mesh (Analabs). When using this column, the chromatograph was programmed to hold at 110 C for 3 min and then to increase at a rate of 4 C/min to 185 C. Myoinositol had a retention time of approximately 15 min.

Radioactive Assay. The scintillation solutions used have been described (17). Phosphorous-32-labeled samples were counted by pipetting an aliquot into a scintillation vial, removing the solvent under a stream of nitrogen, and adding 1.0 ml of ethanol and 15.0 ml of the scintillation solution. An alternate method involved scraping the adsorbent containing the radioactive band into a vial, adding 1.0 ml of ethanol, shaking the vial vigorously, and adding 15.0 ml of scintillation solution. Phosphorous-32-labeled uredospores were solubilized by mild heating (50 C) in 0.5 ml of NCS Solubilizer (Nuclear-Chicago Corp.) before adding the ethanol and scintillation solution. The counting efficiency for 32P-labeled samples was approximately 90%. The counting data for 32P is reported as cpm relative to a standard of the assay. The decay of 32P was taken into account whenever samples counted on different days were compared.

Chemicals. The sources of reference and radioactive compounds were: phosphatidylethanolamine and phosphatidic acid from Pierce Chemical Co., Rockford, Illinois; diphosphatidylglycerol and phosphatidylserine from Nutritional Biochemical Corporation, Cleveland, Ohio; phosphatidylcholine, choline chloride, ethanolamine, myoinositol and ergosterol from Sigma Chemical Co., St. Louis, Missouri; monomethyl ethanolamine and dimethyl ethanolamine from Aldrich Chemical Co., Milwaukee, Wisconsin. Phosphorous-32-labeled phosphoric acid, L-methionine methyl-32C (10.6 mc/mmole), and D,L-serine-32C (4.8 mc/mmole) were from New England Nuclear Corp., Waltham, Massachusetts. All solvents were reagent grade and were used without further purification.

RESULTS AND DISCUSSION

Phospholipids of Quiescent Spores. Thin layer chromatography of the chloroform-methanol extracts of 32P-labeled uredospores yielded, in addition to the origin, five readily observable radioactive components (Fig. 1), which were designated as phospholipids I, II, III, IV, and V. The same regions of the chromatogram could also be visualized by spraying the plate...
with the molybdenum reagent. Ninhydrin reagent indicated that phospholipids II, IV, and V contained ninhydrin reactive groups. Using spores which were uniformly labeled with $^{14}$C, it was determined that the five phospholipids accounted for approximately 3.4% of the spore carbon.

Phospholipid I accounted for approximately 4% of the total lipid phosphorous, and because of its mobility in thin layer chromatography systems it was initially considered to be either diphosphatidylglycerol (cardiolipin), phosphatic acid or a combination of the two phospholipids. When phospholipid I, obtained from ungerminated uredospores, was chromatographed in a system that provides resolution of phosphatidic acid and diphosphatidylglycerol (system C) phospholipid I migrated with standard diphosphatidylglycerol (Table I). Also illustrated in Table I is the agreement of the $R_f$ values of phospholipid I with reference diphosphatidylglycerol, and deacylated phospholipid I with deacylated diphosphatidylglycerol in other solvent systems. The evidence therefore indicated that phospholipid I was diphosphatidylglycerol. However, chromatography of the chloroform-methanol extract of germinated bean rust uredospores indicated the presence of two phospholipid bands in the region of phospholipid I. Furthermore, chromatography of these two bands in the chloroform-methanol-pyridine-2 N ammonium hydroxide system (system C) indicated that phosphate-positive material migrated with both reference diphosphatidylglycerol and reference phosphatidic acid. Therefore, it appeared that during germination a phospholipid with a chromatographic mobility similar to phosphatidic acid was synthesized, or that phosphatidic acid was formed through the action of a phospholipase during the extraction of the germinated spores. The detection of phosphatidic acid in germinating spores, but not in the quiescent spore, is probably due to the more active metabolic state of the germinating spore.

Phosphatidic acid is a key intermediate for phophatidic biosynthesis in mammalian, bacterial, and plant cells and its presence in the germinating uredospore is indicative that phospholipid biosynthesis is occurring (15). However, the presence of phosphatidic acid in phospholipid extracts can be artifactual (15).

Phospholipid II accounted for 23% of the lipid phosphorous. Due to its mobility in chromatography systems and its ninhydrin and phosphate staining properties it was believed to be phosphatidylethanolamine. As shown in Table I, the $R_f$ values of this phospholipid corresponds closely to that of phosphatidylethanolamine in two paper and two thin layer chromatography systems. Furthermore, deacylation of phospholipid II yielded a water-soluble compound which appeared to be identical to the compound formed when reference phosphatidylethanolamine was deacylated under similar conditions. Acid hydrolysis of phospholipid II liberated a ninhydrin-positive compound which exhibited the same chromatographic mobility as ethanolamine in three paper chromatography systems. The agreement between the $R_f$ values shown in Table I for phospholipid II and its derivatives and the $R_f$ values of reference compounds shows that phospholipid II was phosphatidylethanolamine.

Phospholipid III was the major phosphorous-containing component and accounted for 67% of the total lipid phosphorous. Acid hydrolysis of phospholipid III liberated a component which migrated with choline in several chromatography systems (Table I). When phospholipid III and reference phosphatidylcholine were chromatographed in two paper and two thin layer chromatography systems the $R_f$ values were in excellent agreement. In addition, as illustrated in Table I, deacylation of phospholipid III liberated a water soluble component that chromatographed with the water soluble deacylation prod-

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**Table I. Chromatographic Data for the Identification of Phospholipids in Uromyces phaseoli**

<table>
<thead>
<tr>
<th>Component</th>
<th>Rp Values in Chromatographic Systems†</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Diphosphatidylglycerol&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.93</td>
</tr>
<tr>
<td>Phospholipid I&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.93</td>
</tr>
<tr>
<td>Deacylated diphosphatidylglycerol&lt;sup&gt;2, 6&lt;/sup&gt;</td>
<td>...</td>
</tr>
<tr>
<td>Deacylated phospholipid I&lt;sup&gt;1, 6&lt;/sup&gt;</td>
<td>...</td>
</tr>
<tr>
<td>Phosphatidylethanolamine&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.57</td>
</tr>
<tr>
<td>Phospholipid II&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.59</td>
</tr>
<tr>
<td>Deacylated phosphatidylethanolamine&lt;sup&gt;2, 6&lt;/sup&gt;</td>
<td>...</td>
</tr>
<tr>
<td>Deacylated phospholipid II&lt;sup&gt;1, 6&lt;/sup&gt;</td>
<td>...</td>
</tr>
<tr>
<td>Ethanolamine&lt;sup&gt;4&lt;/sup&gt;</td>
<td>...</td>
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<tr>
<td>Ninhydrin positive component of acid hydrolyzed phospholipid II&lt;sup&gt;1, 6&lt;/sup&gt;</td>
<td>...</td>
</tr>
<tr>
<td>Phosphatidylcholine&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.23</td>
</tr>
<tr>
<td>Phospholipid III&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.24</td>
</tr>
<tr>
<td>Deacylated phosphatidylcholine&lt;sup&gt;1, 6&lt;/sup&gt;</td>
<td>...</td>
</tr>
<tr>
<td>Deacylated-phospholipid-III&lt;sup&gt;1, 6&lt;/sup&gt;</td>
<td>...</td>
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<tr>
<td>Choline&lt;sup&gt;5&lt;/sup&gt;</td>
<td>...</td>
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<tr>
<td>N-containing product of acid hydrolyzed phospholipid III&lt;sup&gt;1, 6&lt;/sup&gt;</td>
<td>...</td>
</tr>
<tr>
<td>Phospholipid IV&lt;sup&gt;1&lt;/sup&gt;</td>
<td>...</td>
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<tr>
<td>Phospholipid VII&lt;sup&gt;2&lt;/sup&gt;</td>
<td>...</td>
</tr>
</tbody>
</table>

† Chromatography systems are described and designated in "Materials and Methods."

* Detected with a molybdate reagent.

* Detected by autoradiography.

* Detected by ninhydrin.

* Detected by the method of Marinetti (22).

* Deacylation and hydrolysis procedures are described in "Materials and Methods."

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![Image of the page](https://www.plantphysiol.org/content/48/1/731.full)
uct of reference phosphatidylcholine. Therefore, phosphatidylcholine appeared to be the major phospholipid in uredospores of *Uromyces phaseoli*.

Together, phospholipids IV and V accounted for approximately 5% of the total lipid phosphorous. Chromatography in the chloroform-methanol-water system (system A) indicated both phospholipids to be ninhydrin-positive; however, on rechromatography in system B, only phospholipid V appeared to be ninhydrin-positive. The polar nature of phospholipids IV and V in various thin layer chromatography systems, as well as the polar nature of the deacylation products in chromatography system J indicated that these phospholipids contained inositol. In order to obtain sufficient quantities of phospholipids IV and V the chloroform-methanol extract of the uredospores was chromatographed on preparative thin layer chromatography plates in system A. After extraction from the silica gel, phospholipid IV was further purified by chromatography with preparative thin layer plates using solvent system B. Phospholipid V was not rechromatographed due to the considerable loss of material upon rechromatography. Gas chromatography of the hydrolysis products of phospholipid IV indicated the presence of myo-inositol, but one other as yet unidentified component was also present in the gas chromatographic tracing. An inositol to phosphate ratio of 0.84 to 1.0 was established and phospholipid IV was therefore identified as a phosphatidylinositol. Gas chromatography has also indicated that phospholipid V contained myo-inositol; however, many other unidentified components were present in the chromatographic tracing. A phosphate to inositol ratio in excess of one was observed for this phospholipid. Although phospholipid V was probably an inositol-containing phospholipid, the inositol to phosphate ratio and the presence of a ninhydrin-positive component indicated the molecule was more complex than most phosphatidylinositides.

When the phospholipid compositions of uredospores and the host plant were compared, the most significant difference was the absence of phosphatidylglycerol in the spores. The same relationships exist when the phospholipid composition of the uredospores is compared to that of runner bean plants (15).

The identification of the minor amphipathic lipids of *Uromyces phaseoli* is still in progress.

**Phospholipids of Germinating Spores.** Nonlabeled uredospores, germinated on a medium containing H$_2$PO$_4$ (pH 5.5), incorporated $^3$P into their phospholipids. When such spores were extracted with chloroform-methanol and the extract was chromatographed in chromatography system A, two phospholipids in addition to phospholipids I to V appeared in the autoradiogram. One band migrated slightly ahead of phosphatidylethanolamine and the other migrated between phosphatidylethanolamine and phosphatidylcholine. The mobilities of these compounds in the thin layer chromatography systems and the mobilities of their deacylation products in the paper chromatography system F indicated that these phospholipids were phosphatidylethanolamine, and phosphatidylethanolamine.

Unlabeled uredospores were germinated on a medium containing L-methionine-methyl-$^3$H to determine more precisely the identity of the two phospholipids. Figure 2 shows an autoradiogram of the $^3$H-labeled material when the chloroform-methanol extract was chromatographed on an activated thin layer plate in system D. The four radioactive spots were designated as phospholipids III, VI, VII, and steroid. The relative mobilities of phospholipids VI and VII were in good agreement with those of phosphatidylethanolamine and phosphatidylethanolamine as reported by other authors (31). Phospholipid III exhibited chromatographic characteristics identical to phosphatidylethanolamine which had been previously identified in non-germinated uredospores. The steroids synthesized by this organism appear to be $C_6$ steroids, which are similar to those reported to be present in flax rust uredospores (14).

The $^3$H-labeled phospholipids were identified by extracting the individual bands from the adsorbent and subjecting them to acid hydrolysis. The water soluble $^3$H-labeled products were then chromatographed in two paper chromatography systems. Table II shows that when the radioactive components were chromatographed with the standards dimethylethanolamine, monomethylethanolamine, and choline, the $R_f$ values of the $^3$H-labeled components and the standards were in excellent agreement.

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**Fig. 2.** An autoradiogram showing the thin layer chromatographic separation of the chloroform-methanol extractable, $^3$H-labeled components of *Uromyces phaseoli* uredospores which were germinated on a medium containing L-methionine-methyl-$^3$H. Chromatography was performed with a chloroform-methanol-7.4 N ammonium hydroxide (66:17:3, v/v/v) solvent system. SF, S, and OR. represent the solvent front, a labeled steroid, and the origin respectively. The spots VI, VII, and III were identified as phosphatidylmonomethylethanolamine, phosphatidilmethylethanolamine, and phosphatidylcholine, respectively.
agreement. Therefore phospholipids VI and VII were identified as phosphatidylmonomethylethanolamine and phosphatidyl-dimethylethanolamine. This experiment provided additional evidence for the identification of phosphatidylcholine as a phospholipid in the uredospores of *Uromyces phaseoli*.

Phosphorous-32-labeled phosphatidylserine was not detected in ungerminated bean rust uredospores nor was it detected in germinating spores. However, when uredospores were germinated on a medium containing D,L-serine-3-14C, radioactive components could be extracted from the spores with chloroform-methanol. In addition to the phospholipids, phosphatidyl-ethanolamine and phosphatidylcholine, an autoradiogram of a thin layer chromatogram developed in system B, indicated the presence of a minor band that exhibited the same mobility as phosphatidylserine. Therefore it appears that phosphatidylserine is present in germinating bean rust uredospores, but only in very low concentrations.

The low concentrations of phosphatidylmonomethylethanolamine, phosphatidyl-dimethylethanolamine, and phosphatidylserine were detected by sensitive tracer methods, which involved specific labeling of the compounds. Evidence presented in the succeeding report (18) indicates that phosphatidylmonomethylethanolamine and phosphatidyl-dimethylethanolamine function only as intermediates in the biosynthesis of phosphatidylcholine during the germination of spores, while phosphatidylserine serves as an intermediate for the formation of phosphatidylcholine and phosphatidylethanolamine. Since new membranes are not formed in the resting spore, these three intermediates may not be present. However, very low concentrations of these phospholipids would not have been detected by the methods employed with nongerminated spores.

**Phospholipids of the Germ Tube Wall.** An analysis of the phospholipids in the germ tube wall of *Uromyces phaseoli* uredospores was performed. After the germination of 32P-labeled uredospores on distilled water for 18 hr, the resulting germ tube walls were isolated. The isolation procedure required many aqueous washes before the white flocculent wall preparation was obtained. Extraction of phospholipids was accomplished by the same procedure as that used for the other studies. Thin layer chromatography of the extract in system A was used to separate the mixture into individual components. After autoradiography, to detect bands and identify certain phospholipids, the adsorbent containing each band was scraped from the plate and analyzed by liquid scintillation counting. Table III shows the composition of the germ tube wall in terms of individual components or phospholipids. For comparison, the composition of ungerminated, 32P-labeled spores is given. The composition of the germ tube wall differs from the ungerminated spore as a new component, which had a mobility between that of phosphatidylethanolamine and phosphatidylcholine, was present. This material accounted for approximately 25% of the total phospholipids, and did not appear to be any of the other phospholipids identified in the ungerminated spore or a lysophospholipid. In ungerminated or whole germinated spores this component was present in only trace amounts, presumably, because the concentration of phospholipids in the germ tube wall is low, and the germ tube makes up only a small fraction of the total mass of germinated spores. The difficulty in obtaining sufficient quantities of germ tube walls has slowed our progress in identifying this unusual phosphorous-containing component.

The polar 32P-labeled material which remained at the origin when the extract of ungerminated spores was subjected to chromatography was absent in the phospholipids of the germ tube wall. The relative concentrations of phosphatidylethanolamine and phosphatidylcholine did not differ very much between the two samples, although the percentage of the total phospholipids represented by diphosphatidylglycerol was about three times greater in the germ tube than in the ungerminated spore. It is possible that labeled phosphatidic acid contributed to radioactivity in the diphosphatidylglycerol band since these phospholipids are not resolved by the chromatography system used.

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**LITERATURE CITED**