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A 'nova' in phosphate metabolism, GPG, and discovery of phosphatidylglycerol: a commentary by

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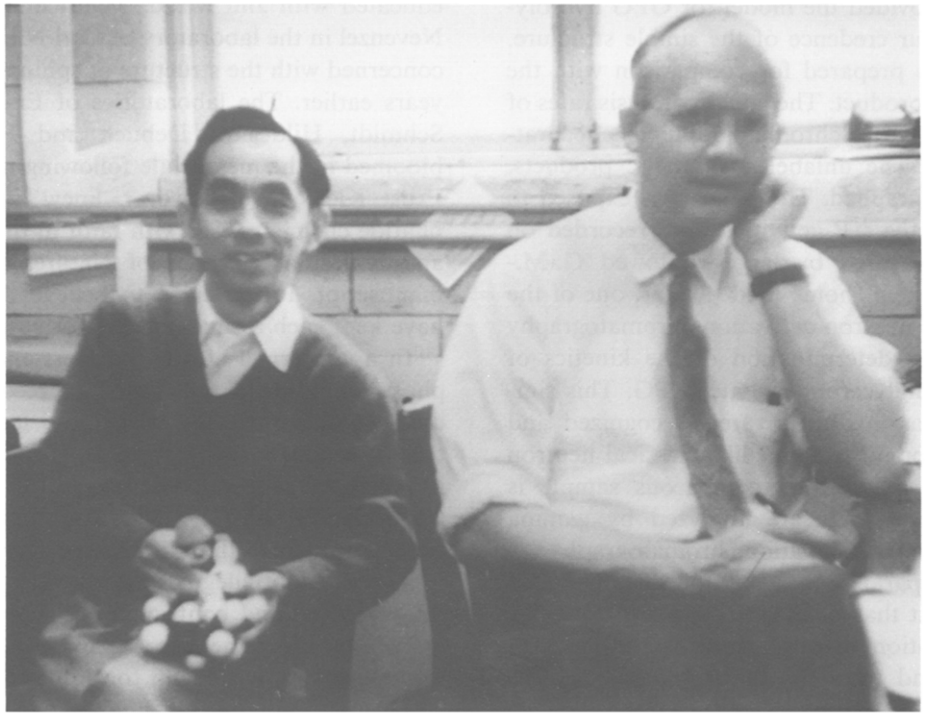
on 'Plant phospholipids. I. Identification of the phosphatidyl glycerols'
by A.A. Benson and B. Maruo
Biochim. Biophys. Acta 27 (1958) 189–195



Bunji Maruo and Andrew Benson

Like a nova in sky where nothing had been before, a novel phosphorus compound appeared in 1955 on radioautographs of two-dimensional paper chromatograms (radiograms) of all plant and algal extracts. In green algae and spinach its concentration exceeded those of the then known intermediates of photosynthesis and sugar metabolism. Its appearance was tantalizing indeed. Like celestial novae which reveal cosmic metabolic processes, the new compound pointed the way to discovery of an important structural and metabolic component whose presence had previously been camouflaged by its simplicity.

The unknown, Up (because it was readily labeled with ^{32}P but only slowly with ^{14}C by photosynthesis), was acid-labile, readily producing a more stable phosphate ester with lower R_F in phenol. About this time, in 1956, Bunji Maruo, then with Shiro Akabori in the Institute of Applied Microbiology of the University of Tokyo, came to our Pennsylvania State University Department of Agricultural and Biological Chemistry. Maruo's stay had been engineered by a good friend Hiroshi Tamiya, leader of Japan's research in photosynthesis. Maruo recognized the problem at once and

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soon had identified the hydrolysis product as GP, glycerophosphate. Clearly, Up was a labile phosphate producing GP upon hydrolysis. To discern the rest of the molecule we labeled algae with ^{14}C and cochromatographed the product with [^{32}P]Up. A small amount of the ^{14}C compound was then eluted and hydrolyzed. It yielded GP, a relatively stable ester, and a product with the mobility of glycerol which we recognized immediately. For novelty we prepared the first, and last, two-color radioautograph of the doubly labeled chromatogram. The ^{32}P side of the film was dyed blue, while the ^{14}C plus ^{32}P side was dyed red. The result was hardly notable.

If glycerol were actually a hydrolysis product of Up, the compound could be diglycerophosphate, GPG [1]. The classic studies of the mechanism of RNA hydrolysis from the Cambridge University laboratory of Alexander Todd provided the model for GPG hydrolysis and enhanced our credence of the simple structure. Synthetic GPG was prepared for comparison with the ^{32}P-labeled natural product. The acid hydrolysis rates of both were measured after chromatographically separating GPG and GP. The unlabeled synthetic products, too, were chromatographed. The paper was exposed to slow neutrons and the ^{32}P activities were recorded on X-ray film and measured by large-windowed G.-M.-counters, many of them 'home made'. Thus, one of the first applications of neutron activation chromatography to biochemistry was determination of the kinetics of acid hydrolysis of diglycerophosphate, GPG. This simple methodology has attributes rarely recognized and less often utilized today. It is not like classical neutron activation analysis, where a heterogeneous sample is activated and the products are analyzed by gamma spectrometry. In neutron activation chromatography the products are first separated on paper and then activated for measurement. At that time, many did not recognize that neutron activation involves immense energies of gamma emission and recoil of the naked phosphorus nucleus followed by a variety of hot-atom interactions with paper, water and solvents.

GPG also had the basic structure of a deacylated phospholipid. After deacylation by a simplified version of the base-catalyzed procedure just developed by R.M.C. Dawson, we chromatographed the products and found GPG in addition to the known diester products. GPG was a major product of deacylation. Could it be a real product or a result of some transesterification? The thought of a lipid as simple as phosphatidylglycerol having been overlooked in 100 years of phospholipid biochemistry was frightening. We could see in the radiograms that GPG was a major phosphate ester of plants and that it was a major product of deacylation of plant lipids. Some, but rather little, occurred in animal tissues. This fact reassured us that the methodology could not be producing the GPG as an artefact.

Our weanling rat with 4 mCi of radiophosphate and its deacylated lipid radiograms provided insight into animal metabolism. Later, we could do the same kind of analysis without radiophosphate. We deacylated bovine heart muscle mitochondrial lipids, sheep liver lipids, et al. and separated them on our two-dimensional paper chromatograms. After applying μg spots of phosphate on the edges, the papers were rolled, sealed in polyethylene tubes and irradiated with slow neutrons in the pool by the Penn State nuclear reactor. The ^{31}P became ^{32}P and the induced radioactivities could be compared to that induced in the adjacent standards, giving quantitative values for each of the parent phospholipids.

Sending the manuscript to the editors of BBA was a fearful experience. Could such a simple compound of glycerol, phosphate and fatty acids be real? We were amateurs in lipidology and knew it. Though I had been educated with Jim Mead, Maurice Rapport and Judd Nevenzel in the laboratory of Carl Niemann at CalTech, concerned with the structure of sphingosine, that was 20 years earlier. The laboratories of Erich Baer, Gerhard Schmidt, Hildegard Debuch and Herb Carter had bloomed in the meanwhile following the elegant trail of Ernst Klenk. We, however, knew little of real phosphatide chemistry. So, with faith in the logic, simplicity and molecular elegance of Nature, we submitted the manuscript. It was hard to believe that Nature could have kept such a simple secret for so long.

In a concerned effort to understand if and how the phospholipid field had failed to recognize phosphatidylglycerol I conferred with those leading research in that direction. James Lovern at Torry Research Station in Aberdeen had observed glycerol in his phospholipid fractions. He and June Olley had made sound observations pertinent to the existence of phosphatidylglycerol. I enjoyed the privilege of meeting Dr. Lovern in beautiful Aberdeen and confirmed our interpretations of his work. On the same trip I met with Professor James Baddiley, who had begun to unravel the chemistry and structures of the teichoic acids, polymers and glycerophosphate and ribitol phosphate, and with his colleague Grant Buchanan, also a superb carbohydrate chemist in the Newcastle Department. We discussed nomenclature of glycerophosphate and ribitol phosphate.

The nomenclature polemic: 'D-glycerol 1-phosphate' or 'L-glycerol 3-phosphate' – Emil Fischer had guessed right! At a 1952 conference at Penn State, J.M. Bijvoet had reported application of anomalous diffraction of X-rays by heavy nuclei with the consequent proof of Fischer's presumption. In my discussions with Baddiley and Buchanan and later with Hermann O.L. Fischer it was clear that real carbohydrate chemists considered it entirely appropriate to assign the term 'C-1' to the carbon bearing a substituent such as phosphate ester which rendered a symmetrical molecule asymmetric and

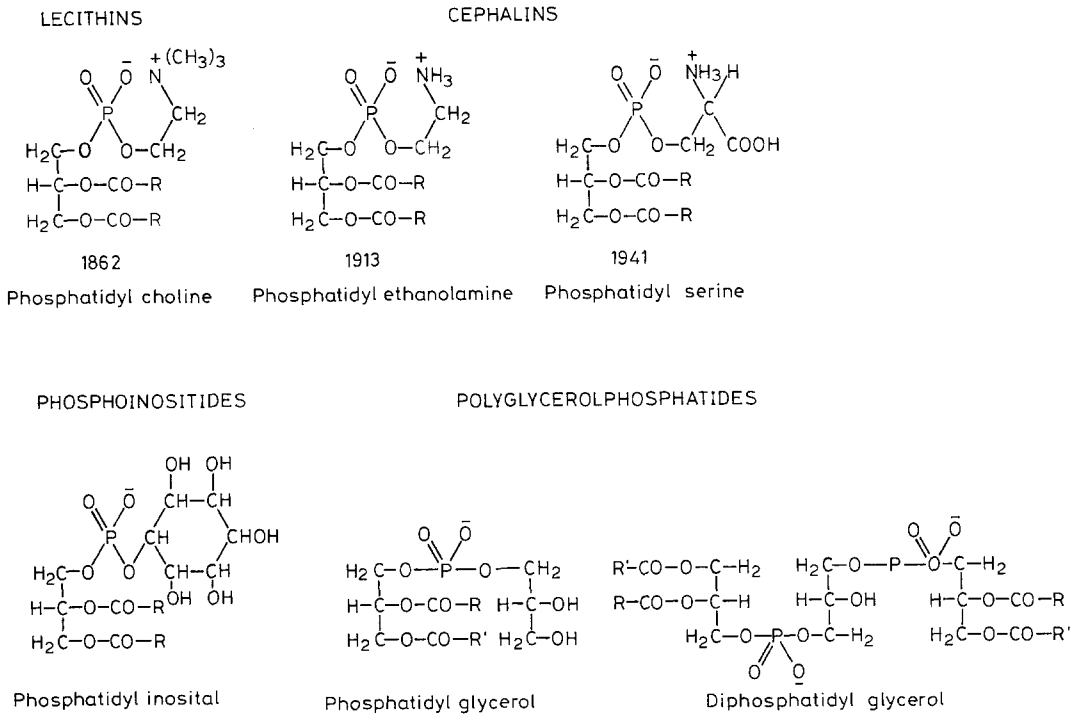

Fig. 1. The glycerolphosphatides.

optically active. Thus, natural glycerophosphate becomes D-glycerol 1-phosphate. There was no a priori reason to consider using an L-glycerol convention without some very good reason. Thus, phosphatidylglycerol was written 'rightside-up' in our paper and not downside-up as it is now in contemporary texts. The result was a neat, compact, readily printed and easily remembered stereo representation of phosphatidylglycerol (Fig. 1).

But, Erich Baer thought in other terms. His ester, 'Fischer-Baer Ester' was 'D-glyceraldehyde 3-phosphate' – because to him the important aspect was its 'sugar' character and its phosphate ester fell on C-3. That was fine. But Baer insisted on going further. In his imagination and later chemically (but not enzymatically) the Fischer-Baer Ester could be prepared from the enantiomer of natural glycerophosphate by oxidation of D-glycerol 3-phosphate! This relationship was so real in his mind that the natural glycerophosphate could only be termed 'L-glycerol 3-phosphate', which he called L-α-glycerophosphate, the phosphate being on an end carbon atom. Publishing so many elegant reports of his glycerolphosphatide syntheses, Baer's 'convention' found its way into the literature and the committees of the IUB. He could never accept the simplicity of the alternative. One ventures to think that the ghost of Emil Fischer must have cringed at the thought.

The L-α-glycerophosphate convention prevailed and consequently led to the upside down structures we see in the texts today (Fig. 2). Current convention, which could have led to a dextro-, D-, or *r*-name, sealed the fate of rightside-up glycerophosphate with the '*sn*-3-glycero-' nonenclature we now see, a translation of L-glycerol 3-phosphate. This was a pitiful unsimplification which sanity could have avoided. Acres of printed structure space could have been saved and countless examples of CHOAc or C-CHOAc-C in texts, failing to give students a clear impression of the very simple stereostructure, could have been avoided. An obvious convention could have been adopted by which D- and L-glycerol could be used when a substituent confers asymmetry at C-2.

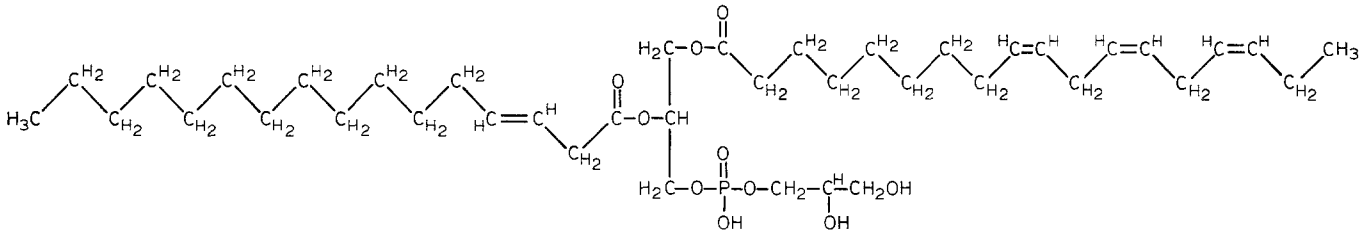

Fig. 2. Phosphatidylglycerol.

At the outset we knew of Baddiley's CDP-glycerol and the likelihood that it could be involved in phosphatidylglycerol biosynthesis. In 1956 we had no real information on the stereostructure of the lipid's two glycerols and incorrectly presumed both were the same. It was the elegant work of E.P. Kennedy that demonstrated the reality of the phosphatidylglycerophosphate intermediate in liver and the true mechanism of biosynthesis. The configuration of both glycerols was then clear.

In later work, Robin Prasad [2] studied the products of bacterial GPC phosphodiesterase hydrolysis of GPG. He determined that the GP produced was D-glycerol 1-phosphate, as with other glycerophosphoryl esters derived from phospholipids. Again, L-glycerol 1-phosphate did not appear as a natural product, even though its relationship to D-glyceraldehyde 3-phosphate had been envisioned.

A second tantalizing unknown revealed itself from time to time in our ^{32}P-labelled lipid hydrolysates. Another GP derivative! The unknown was easily hydrolyzed to produce GP by a few minutes in dilute acid at 24° C. In fact, Dowex 50 · H^+ was observed to produce the unknown as well as to hydrolyze it to yield GP. Bunji Maruo worked out these relationships, which were modeled by Alexander Todd's studies of RNA hydrolysis and Jim Baddiley's studies of teichoic acid hydrolysis in which cyclic phosphate esters had been found as transesterification by-products. The unknown was glycerol 1,2-cyclic phosphate [3], an artefact of the deacylation procedure and derived largely from glycerophosphorylcholine.

From time to time, a novel diester appeared among the deacylated lipids of algae or leaves. It, too, hydrolyzed to yield GP. Its sporadic appearance in 1955–58 made it difficult to attack. It wasn't until 1964 that Sofia Freer's fortunate error led to recognition of the transphosphatidylation action of phosphospholipase D [4]. She had collected Chinese cabbage instead of spinach in a local field. Its white vascular tissue appeared to be rich in phosphatidylmethanol, that being the solvent used for primary extraction. Other alcohols produced corresponding lipids. Shang Fa Yang examined the substrate specificity of the enzyme for the transphosphatidylation. Ethanolamine and glycerol were

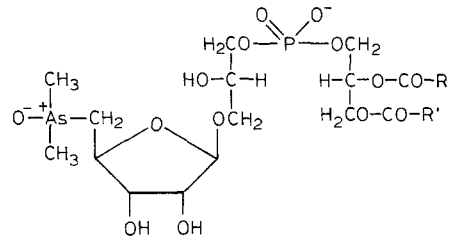

Fig. 4. Phosphatidylglycerol-3′-(5″-dimethylarsenoso, 5″-deoxyriboside).

excellent acceptors. We reported our findings at the 9th International Conference on Biochemistry of Lipids in Nordwijk on September 7, 1965. This enzymatic synthesis of phosphatidylglycerol interested Rex Dawson, who then recognized the relation of our results to his observations in glycerol-containing media. Our publication finally appeared in February 1967, about the same time as Dawson's. In time, phospholipase D transphosphatidylation has become a method of choice for preparation of PG and related lipids.

Phosphatidylglycerol, like its inositol analog, is an 'acidic' or calcium/magnesium-binding membrane lipid. In spite of its high concentrations in plant and bacterial membranes, its functions remain obscure. The discovery of a unique phosphatidylglycerol containing a 2-substituted Δ3-*tr*-hexadecenoic ester by Haverkate and Van Deenen [5] (Fig. 3) offered promise of revealing a specific function in chloroplast lamellar membranes. Thus far, its function has not been established. Genetic manipulation of *E. coli* membrane lipid synthesis by Isao Shibuya and his colleagues [6] resulted in remarkable syntheses of phosphatidylmannitol and analogous lipids derived from glycitols added to the culture media. Alteration of membrane function in such mutants offers promise for recognition of specific functions for membrane lipids. Recognition of arsenical and stibnyl derivatives of phosphatidylglycerol resulted from elucidation of the arsenical metabolites of arsenate by aquatic algae and plants [7,8] and study of their lipid derivatives. As such, the lipids appear to mediate excretion of arsenic and antimony (Fig. 4).

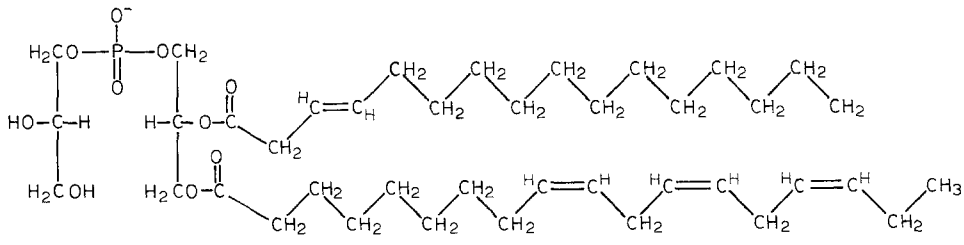

Fig. 3. Phosphatidylglycerol of chloroplast membranes.

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PLANT PHOSPHOLIPIDS

I. IDENTIFICATION OF THE PHOSPHATIDYL GLYCEROLS*

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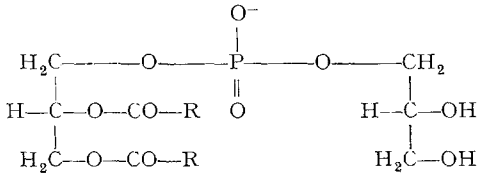
The identification of α, α'-diglycerophosphate in *Scenedesmus*[1,2] and of cytidine diphosphate glycerol[3] in *Lactobacillus arabinosus* suggested the possible existence of the phosphatidyl glycerols. These compounds could be formed by a reaction exactly analogous to that demonstrated by KENNEDY AND WEISS[4] for the biosynthesis of the lecithins and would possess the structure



A major point of confusion in the phospholipid literature rests with the difficulties inherent in identification of partially purified lipids which have little or no nitrogen. The existence of the phosphatidic acids in nature was first questioned by HANAHAN AND CHAIKOFF[5] and their contention has recently been verified by KATES[20]. MARINETTI reported absence of phosphatidic acids in tissues. That there has existed an important non-nitrogenous component besides the inositol lipids is almost clear from the remarks of a host of workers and reviewers.

Small amounts of phospholipids containing polyglycerophosphate have been reported by PANGBORN[7] (cardiolipin), FLEURY[8] (glycérophosphatogène), MCKIBBIN AND TAYLOR[9] (polyglycerolphosphatide) and IGARASHI *et al.*[10]. PANGBORN had considered the possibility of a bis-glycerophosphatidic acid structure for cardiolipin. Interest in the properties of these lipids led BAER[11] to prepare several enantiomeric α-bisphosphatidic acids.

The existence of the phosphatidyl glycerols appears to have been overlooked because the simplicity of their structure and the identity of their degradation products with those of other glycerolphosphatides produced no profound changes in phospholipid analytical results. A correlation of the properties of fractionated lipids and the nature of the glycerophosphoryl esters resulting from their degradation has led to the identification of this group of glycerolphosphatides.

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MATERIALS AND METHODS

Lipids of Scenedesmus-^{32}P

Scenedesmus cells (0.5 g) were allowed to grow 24–72 hours at 1000 ft.-c. in 50 ml of culture medium containing no phosphorus other than that in 3 mc (0.1 mg) radiophosphate. The cells were washed with water and four volumes of hot absolute alcohol was added to the packed cells. The 80% alcohol extract was chromatographed two-dimensionally on unwashed Whatman No. 1 or No. 4 paper using phenol–water and butanol–propionic acid–water solvents[12]. The ^{32}P-lipid components separated into four distinct spots the two fastest possessing the greatest radioactivity (Fig. 1, Table I).

Phospholipids-^{32}P of leaves

Excised leaves of tobacco, barley, sweet clover (*Melilotus alba*) and of white clover (*Trifolium repens*) were illuminated for 15 hours with their stems in a neutral solution of radiophosphate (1 mc/ml). The stems were rinsed with water and the leaf plunged into hot 95% ethanol. Further extracts with 80% and 100% ethanol were combined and concentrated to 0.2 ml. Aliquot samples were chromatographed on Whatman No. 4 paper. The lipid areas were eluted with pyridine and evaporated to dryness at room temperature.

Deacylation of ^{32}P-lipids

After removal of the pyridine the lipids were taken up in 50 μl ethanol containing 5% carbon tetrachloride. To this solution was added 100 μl of 0.2 N KOH in methanol[13]. After 20 minutes or 5 hours at 37° an equivalent amount of dry Dowex-50-H$^+$ was added. Addition of a drop of water and ten seconds stirring gave a neutral solution suitable for chromatography. The cation exchange resin which was washed with ethanol to remove residual lipids or products did not adsorb appreciable amounts of GPE or GPC*.

Chromatography of the glyceryl phosphoryl esters

Using the same solvents systems with unwashed filter papers the products of hydrolysis were easily separated with R_F values given in Table I. All of the lipid ^{32}P was found in these compounds. No detectable orthophosphate or glycerophosphate was formed. Phospholipid analyses were performed by direct counting of hydrolysate chromatograms such as that for tobacco leaf lipids shown in Fig. 4.

Lead tetraacetate oxidation of phosphatidyl glycerols

To the eluate of ^{32}P-lipid II in acetic acid solution was added 2 mg of freshly prepared lead tetraacetate and an equivalent amount of ethanol. After one hour an equivalent amount of ethylene glycol was added. Water was added and the lipid material taken up in chloroform. The chloroform solution was evaporated to dryness and the residue product hydrolyzed as before. Chromatography of the deacylated product gave approximately equal amounts of GP and a product with considerably higher R_F in phenol–water and equal R_F in butanol–propionic acid–water than that of GPG.

Benzoylation of phosphatidyl glycerols

A sample of the eluted phosphatidyl glycerols was tested for free hydroxyl groups by benzoylation with benzoyl chloride in pyridine solution. After two minutes at forty degrees the reaction mixture was diluted with water and extracted with petroleum ether. After evaporation of the solvent, 100 μl of 0.2 N KOH in methanol was added and allowed to stand 30 minutes at 37°. Chromatography of the decationized product revealed a major component with R_F = 0.93 in phenol-water and R_F = 0.92 in butanol–propionic acid–water which was neither the original lipid nor GPG and had the mobility anticipated for a dibenzoyl ester of GPG. A second component of the hydrolysate contained 30% of the total ^{32}P and had an R_F in phenol of 0.70 which suggested its identity as γ-benzoyl-α, α'-diglycerophosphate. Each benzoylated product was hydrolyzed separately in 1.0 M methanolic potassium hydroxide at 70° for one hour. The solutions were decationized with excess Dowex-50-H$^+$ and chromatographed in phenol–water. For each hydrolysis the products were found to be 70% GPG and 30% GP.

Acetonation of phosphatidyl glycerols

A sample of the phosphatidyl glycerols was tested for vicinal hydroxyl groups by reaction with dry acetone in the presence of hydrogen chloride. The labeled lipid in 0.5 ml acetone containing 1% HCl was allowed to stand two days at room temperature. The sample was then allowed to evaporate

* The following abbreviations will be used in this article: CTP, cytidine triphosphate. CMP, cytidine monophosphate. CDPGlycerol, cytidine diphosphate glycerol. GPG, glycerophosphorylglycerol. GPC, glycerophosphorylcholine. GPE, glycerophosphorylethanolamine. GPInos, glycerophosphorylinositol. GP, L-α-glycerophosphate = D-glycerol-1-phosphate. POP, pyrophosphate.

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slowly in a desiccator over KOH. The residue was taken up in ethanol (0.05 ml) and treated with 0.1 ml of 0.2 *N* methanolic KOH for 30 minutes at 37°. Without acidification an aliquot portion of the solution was chromatographed in phenol–water solvent on Whatman No. 4 paper. The new radioactive product, R_F = 0.89 was observed. This product was eluted and treated with Dowex-50-H^+ at 60° for ten minutes. Cochromatography in phenol–water demonstrated the identity of the hydrolysis product with GPG.

RESULTS AND DISCUSSION

Chromatography of *Scenedesmus*-^{32}P extracts separated the phospholipids into four distinct groups shown in Fig. 1. Lipids I and II do not separate in phenol but can be resolved in the butanol-propionic acid-water solvent. Approximate R_F values and total ^{32}P activities of these groups are given in Table I. Refined techniques[14] may separate these more completely and reveal minor components. Extracts of *Melilotus alba* and of *Trifolium repens*, two species of clover, gave similar results but with only the three major P-lipid areas. The phosphatidyl ethanolamines and the phosphatidyl serines occurred in smaller amounts and were estimated by determining the ^{32}P activity in glycerophosphorylethanolamine and glycerophosphorylserine obtained on hydrolysis of eluates of the total lipid areas from chromatograms.

TABLE I

R_F VALUES OF *Scenedesmus* PHOSPHOLIPIDS AND THEIR HYDROLYSIS PRODUCTS

	Phospholipids			*KOH Hydrolysate*		
	% Total	*R_F Phenol**	*R_F Bu-Pr*	*R_F Phenol*	*R_F Bu-Pr*	*Identified as*
I	36.1	0.90	0.94	0.89	0.29	GPC
II	41.2	0.87	0.85	0.40	0.17	GPG
III	14.6	0.73	0.68	0.12	0.05	GPInos
IV	9.0	0.57	0.59			(GPInos)**
	1.0			0.22	0.12	GPSer***
	6.1			0.65	0.23	GPE***

* R_F values for *Scenedesmus* phospholipids on Whatman No. 4 unwashed paper and calculated for the center of density of the spots. Chromatograms were descending type developed with (1) 75% phenol and (2) butanol–propionic acid–water.

** Lipid IV was only 10% hydrolyzed. The product was GPInos.

*** Determined from hydrolysate of total lipids.

The lipids I, II and III were classified according to their products of deacylation which showed them to be surprisingly homogeneous (Fig. 2, Fig. 3). R_F values for these products are given in Table I. Minor hydrolysis products could be accounted for by contamination with material from adjacent spots. The composition of phospholipids of *Scenedesmus*-^{32}P cells and of leaves of tobacco^{32}P, barley-^{32}P and clover-^{32}P are given in Table II.

It is seen that the lecithins have the highest R_F and concentrations and are followed in both respects by the phosphatidyl glycerols. This observation immediately precluded the existence of large amounts of α-bisphosphatidic acids which would have higher R_F's than either the lecithins or the phosphatidyl glycerols. Further experiments to determine the structure were performed. The phosphatidyl glycerols were oxidized with lead tetraacetate and hydrolyzed to give a product, glycerophos-

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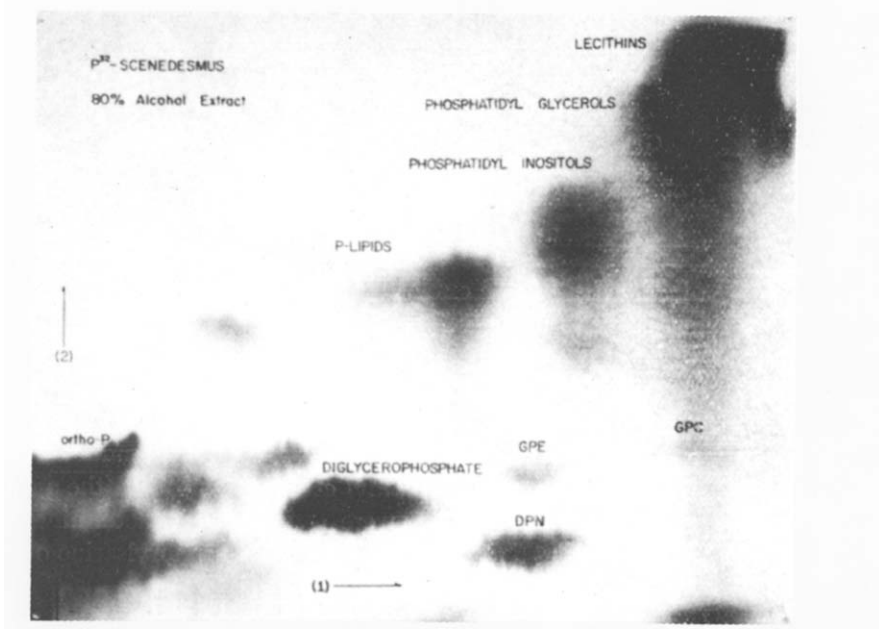

Fig. 1. Radiogram of alcohol extract of *Scenedesmus* labeled with radiophosphate. Chromatogram was developed in direction (1) with phenol–water and in direction (2) with butanol–propionic acid water.

TABLE II

COMPOSITION OF PLANT PHOSPHOLIPIDS

Phospholipid	*Scenedesmus* %	*Tobacco* %	*Sweet clover* %	*Barley* %
Lecithin	36.1	46.5	30.0	52.4
Phosphatidyl glycerol	41.2	22.0	24 9	22.6
Phosphatidyl inositol	14.6	22.4	17.5	14.0
Phosphatidyl ethanolamine	6.1	7.9	8.6	1.4
Phosphatidyl serine	1.0	0.7	6.3	—

phorylglycolaldehyde which was shown to be different from GPG by failure in cochromatography. The phosphatidyl glycerols were benzoylated and then deacylated to give a benzoate of diglycerophosphate. An acetone derivative of the phosphatidyl glycerols was prepared and deacylated to give a new compound with chromatographic properties anticipated for those of monoisopropylidene-α,α'-diglycerophosphate. These observations indicate that the naturally occurring lipids are phosphatidyl glycerols rather than α-bisphosphatidic acids.

Hydrolysis of P-lipid III from each of the plants examined gave pure glycerophosphorylinositol the structure of which is not yet rigorously proved. Its acid hydrolysis products provide evidence for its structure as named. It was demonstrated by TODD AND BROWN[15] that α-hydroxy diesters of phosphoric acid hydrolyze by cyclization to an intermediate 1,2-cyclic phosphate followed by cleavage. By such a process one would expect glycerophosphorylinositol to yield more than twice as much inositol phosphate than α-glycerophosphate since the two α-hydroxyl groups of inositol are forced closer to the phosphorus atom than is the α-hydroxyl group of glycerol. After

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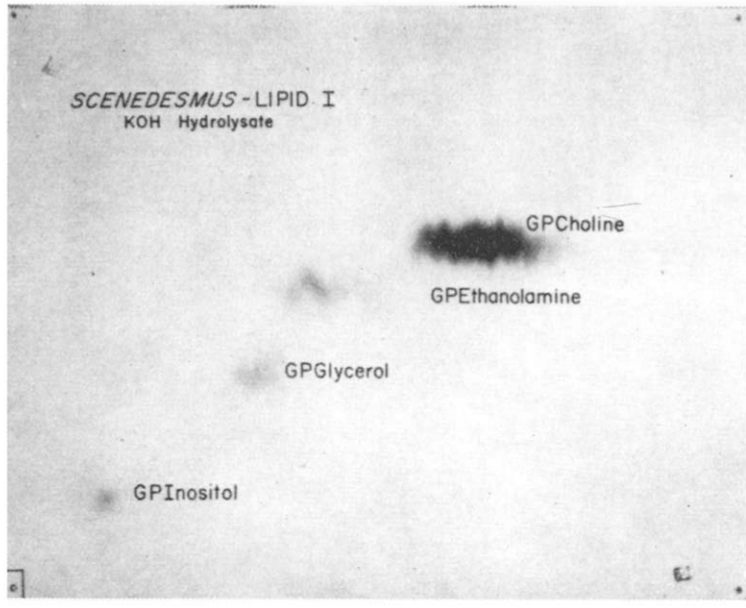

Fig. 2. Methanolic KOH hydrolysate of Lipid I.

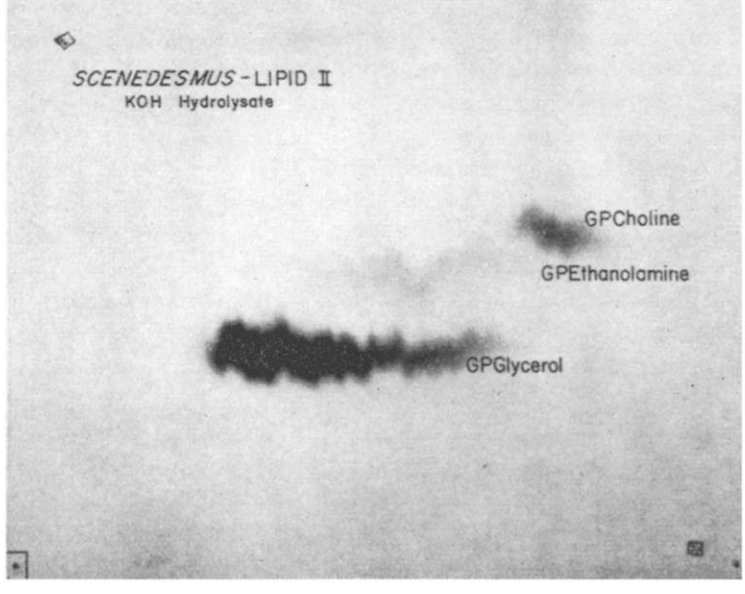

Fig. 3. Methanolic KOH hydrolysate of Lipid II.

ten minutes at 100° in 1 *N* hydrochloric acid the hydrolysate of this ester was chromatographed and the two products counted. The ratio of inositol phosphate to glycerophosphate was 2.95:1. These observations are in accord with the α-phosphatidyl inositol structure proposed by FAURE AND MORELEC-COULON[16] for a wheat germ lipid and with the results reported by WAGENKNECHT AND CARTER[17] for a phosphatidyl inositol of peas.

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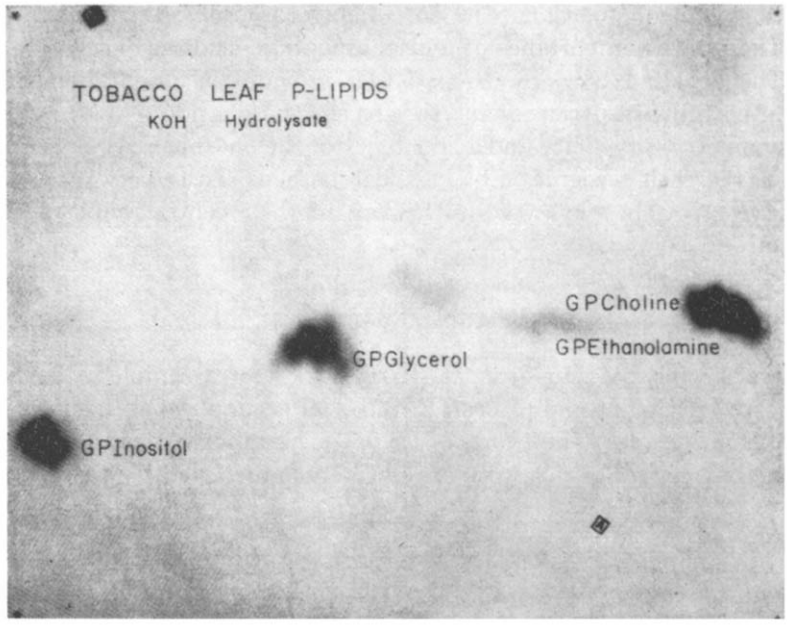

Fig. 4. Methanolic KOH hydrolysate of total phospholipids of tobacco leaf.

The amount of glycerophosphate formed during any of these hydrolyses was scarcely detectable. It is clear that phosphatidic acids do not exist in the tissues studied so far. Further it is now even more apparent that a major fraction of phosphatidic acids previously reported arise as postulated by HANAHAN AND CHAIKOFF[5] as a result of action of phosphatidase C or by action of mineral acid during purification procedures. The acid hydrolysis of GPG is very rapid. The half time in 1.0 N HNO_3 at 100° was found to be close to two minutes[2]. Evaporation of hydrochloric acid solutions of GPG at paper chromatogram origins resulted in extensive hydrolysis. This treatment presumably corresponds to treatment of the GPG with 6 N hydrochloric acid at room temperature for half an hour. This rate appears to be somewhat higher than that reported by SCHMIDT *et al.*[19] for GPE and should be anticipated for a bis-α-hydroxy ester of phosphoric acid.

The phytosynthesis of the phosphatidyl glycerols probably follows the pathway established for lecithin and phosphatidyl ethanolamine by KENNEDY AND WEISS[4]. The series of reactions would be represented by the following equations:

$$\text{Dihydroxyacetone phosphate} \xrightarrow[\text{DPNH}]{\text{"}\alpha\text{-Glycerophosphate dehydrogenase"}} \text{D-Glycero-1-phosphate}$$

$$\text{D-Glycerol-1-phosphate} + \text{CTP} \xrightarrow{\text{"Phosphoglycerol cytidylyl transferase"}} \text{CDPGlycerol} + \text{POP}$$

$$\text{D-2,3-Diglyceride} + \text{CDPGlycerol} \longrightarrow \text{Phosphatidyl glycerol} + \text{CMP}$$

The symmetry of diglycerophosphate suggests that some reservations may be necessary in considering this mechanism and that it may play a more important role in lipid biosynthesis than is immediately apparent. The concentration of free GPG in *Scenedesmus*[2] can be extremely high ($10^{-3}M$) but decreases rapidly during photo-

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synthesis. Experiments to evaluate its potentialities as a substrate for acylation are in progress. The possible importance of α-bisphosphatidic acids as diglyceride donors is obvious. The acylated GPG lipids may well provide the orientation essential in synthesis of both the triglycerides and the glycerolphosphatides.

The symmetry, simplicity and acid lability of the phosphatidyl glycerols appears to have delayed their recognition by classical methods. These very properties of the phosphatidyl glycerols may well be the key to their central importance in lipid metabolism.

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SUMMARY

1. The phospholipids of *Scenedesmus* and higher plants have been separated into at least three fractions by two-dimensional paper chromatography. These have been deacylated to yield glycerophosphorylcholine, glycerophosphorylglycerol and glycerophosphorylinositol.

2. The phosphatidyl glycerol fraction has been identified by hydrolysis of the purified lipid to give glycerophosphorylglycerol in 95% yield.

3. The presence of two free hydroxyl groups in the phospholipid was demonstrated by its conversion to an isopropylidene derivative and by oxidation by lead tetraacetate.

4. These lipids possess almost half of the lipid phosphorus in some plants and appear to be ubiquitous in nature.

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