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Inositol Phosphatides—Structure, Biochemistry, Synthesis

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Progress made during the past 10-12 years in establishing the structure, elucidating the biological role, and synthesising the inositol phosphatides—a class of phospholipids—is discussed. The first part considers problems involved in establishing the structure of mono-, di-, and tri-phosphoinositides, mannosides of phosphatidylinositol, and more complicated "inositide" complexes. The second part deals with the biochemistry of inositol phosphatides, in particular the properties and functions specific to phosphoinositides. The problems and successes in the synthesis of phosphoinositides are discussed in the last part. A large amount of experimental information is used to show the complexity of the selective protection of the hydroxy-groups in myoinositol with the aim of obtaining initial compounds for the synthesis of inositol phosphatides. Work done during recent years is shown to open up a path to the synthesis of optically active phosphoinositides of natural structure. A list of 114 references is included.

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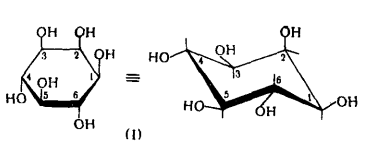
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I. INTRODUCTION

The inositol phosphatides are among the most important examples of phospholipids. The presence of phosphoinositides in Nature was first pointed out by Anderson[1], who isolated them from tubercle bacilli. It has now been found that phosphoinositides are present in plants, various animal organs, and micro-organisms: they have been found in soya beans[2], kidney beans[3], milk[4], the brain[5], the liver, kidneys, and other sources[6]. Two reviews have been published dealing in some measure with problems of the structure and synthesis of inositol phosphatides[6,7]. As our knowledge of these compounds becomes deeper and broader, new aspects of their biological role and chemical properties are continually opening up, showing the extraordinary importance of these compounds in the life of various organisms. The present review deals briefly with recent work on the chemistry and the biochemistry of the inositol phosphatides.

II. COMPONENTS AND STRUCTURE OF INOSITOL PHOSPHATIDES

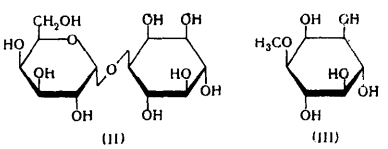
A structural unit of all inositol phosphatides is myoinositol (MI) (I), the only inositol present in Nature in the form of phosphate derivatives[8].



(I)

Myoinositol is widely distributed in Nature, both in the free and in the combined form. It has been found, for example, in rubber latex[9], sperm plasma[10], and sugar beet[11]. It occurs in these sources together with galactin[12], 1-*O*-α-*D*-galactopyranosyl- (1*R*)-myoinositol (II). (Here and subsequently the configuration in asymmetrically substituted derivatives of myoinositol is given in Angyal's notation[13]).

Mention must be made also of such natural myoinositol compounds as (+)-bornesitol[14] or 1-*O*-methyl-(1*R*)-myoinositol (III), α-*D*-galactopyranosyl-(1→6)-α-*D*-galactopyranosyl-(1→1)-myoinositol[15], isolated together with galactin from the roots of the pea, and various myoinositol mannosides, the structure of which will be discussed below.



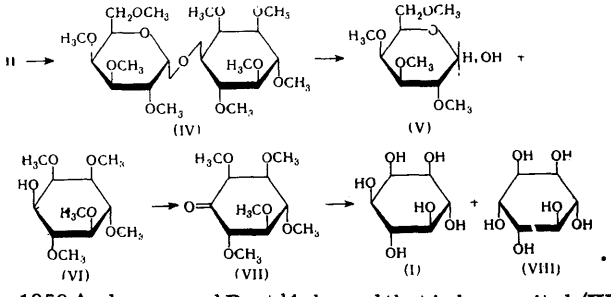
(II) (III)

Sugars and amines must be noted among other components of inositol phosphatides, besides the phosphates, glycerol, and fatty acids usually met in their hydrolysis products. Not long ago many investigators regarded sugars and amines as "impurities" in inositol phosphatides, and endeavoured to remove them by chromatographic and chemical methods of purification[16]. Such "purification" probably resulted frequently in breakdown of the complicated native inositol phosphatide molecule[17]. Anderson[18] showed in several papers that mannose is present in inositol-containing glycolipids of tubercle bacilli. Arabinose and galactose have been found in purified phosphoinositides of soya-beans[19] and lipids of groundnuts[20]. D-Glucose, combined with myoinositol by a glycosidic bond, has been isolated also from the glycolipids of acid-stable bacteria[21]. In 1965 Galanos and Kapoulas worked out a technique for isolating lipid fractions[22], on the basis of which they suggested that lipids, including inositol phosphatides, are present in vegetable and animal tissues as complicated triesters of phosphoric acid[23]. Sugars may also be components of these "triester complexes".

Nitrogen bases also are effective components of inositol phosphatides. Thus aminoethanol has been detected in inositol phosphatides from groundnuts[20], and soya-beans, and in the glycolipid of acid-resistant bacteria. Choline, aminoethanol, and serine may form part of the "triester complexes"[22,23]. Amino-acids—L-ornithine[24], hydroxylysine, asparagine, glutamine, glycine, alamine, etc.—have been observed in many inositol phosphatides[3]. Thus inositol phosphatides containing glutamic and aspartic acids, valine, leucine, threonine, and isoleucine have been isolated from wool fat[25].

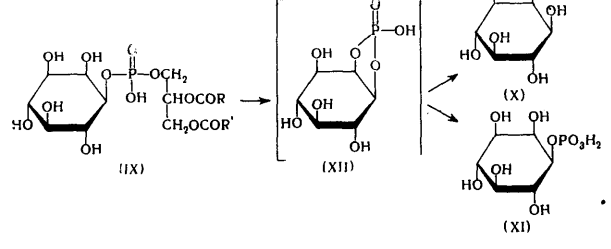
In 1956 the structure of the simplest inositol phosphatides had still not been established; in particular, such an important question as the point of addition of phosphate to myoinositol was obscure[6]. Most workers considered that the phosphate residue was bound to an axial 2-hydroxyl of the myoinositol, since hydrolysis of inositol phosphatides yielded the 2-phosphate.

In 1953 Kabat et al.[12] had established that in galactin (II) a galactopyranose residue was combined by means of an α-glycosidic bond with the 1-hydroxy-group of myoinositol. Methylation of (II) with dimethyl sulphate gave nonamethylgalactin (IV), hydrolysis of which in 0.5 *N* hydrochloric acid yielded 2, 3, 4, 6-tetramethyl-D-galactose (V) and the pentamethyl ether of myoinositol (VI). The latter was oxidised with chromium trioxide to myoinosose (VII), reduction of which followed by demethylation gave both myinositol (I) and (–)-inositol (VIII), which proved the structure given here for galactin (II):



In 1958 Anderson and Post[14] showed that in bornesitol (III) the 1-hydroxy-group of myoinositol had been methylated.

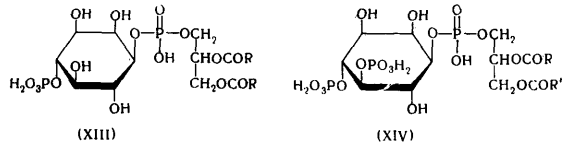
In contrast to these natural compounds of myoinositol, it was very difficult to establish the position of the myoinositol–phosphate bond in phosphoinositides, owing to migration of the phosphate residue during hydrolysis of the natural lipids. The myoinositol molecule (I), having a plane of symmetry through positions 2–5, is optically inactive, but introduction of a substituent into position 1, 3, 4, or 6 should result in the appearance of optical activity. In 1959 several papers were published[26-28] which showed that the hydrolysis of natural phosphoinositides, e.g. phosphatidylinositol (IX), yields a mixture of 2– and 1-phosphates of myoinositol (X) and (XL), which are formed by phosphate migration under conditions of hydrolysis, with intermediate formation of a cyclic ester (XII):



Since the myoinositol 1-phosphate obtained (XI) was optically active, the intermediate cyclic phosphate (XII) should be optically active, which would have been impossible if the myoinositol in the natural compound (X) had been phosphorylated in position 2. This demonstrated that the phosphate residue in natural phosphoinositides is attached to the $C_{(1)}$ atom of the myoinositol molecule.

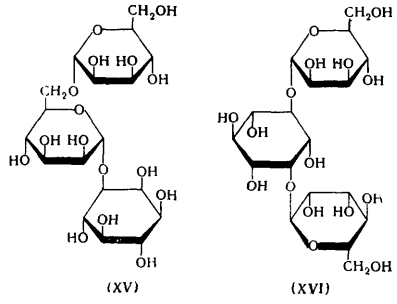
This was confirmed in another paper[27]: glycerylphosphorylinositol, isolated from cow's liver ($[\alpha]_D^{25}$ –13.5° for the cyclohexylammonium salt) yielded after periodate oxidation the optically active glycolaldehydemyoinositol phosphate ($[\alpha]_D^{25}$ –13.2° for the cyclohexylammonium salt: i.e. the main contribution to the optical activity of the natural compound is made by the asymmetrically substituted myoinositol 1-phosphate). Thus in galactin (II), bornesitol (III), and phosphoinositides of soya-beans[29], liver[30], and other sources, myoinositol is substituted in the (equatorial) position 1.

During recent years several major laboratories (those of Ballou, Hawthorne, Dawson, etc.) have been conducting intensive research into polyphosphoinositides possessing great metabolic activity and important biological functions. The structures (XIII) and (XIV) have been established[31-33] for di- and tri-phosphitides:



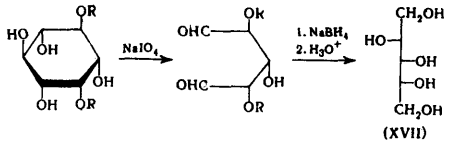
Periodate oxidation of myoinositol triphosphate, isolated from products of the alkaline hydrolysis of the triphosphoinositide of bull's brain, with subsequent reduction and dephosphorylation, gave D-iditol. Similarly, D-threitol was obtained from the diphosphate[32]. From this it was established that the triphosphoinositide was the 4,5-diphosphate of 1-phosphatidyl-(1*S*)-myoinositol (XIV), and the diphosphoinositide the 4-phosphate of 1-phosphatidyl-(1*S*)-myoinositol (XIII)[33].

Certain glycolipids of acid-resistant bacteria belong to an interesting class of inositol phosphatides. Anderson showed that these lipids contain a compound of myoinositol with two molecules of mannose, which was termed manninositose[34]. In 1960 Vilkas and Lederer[35] hydrolysed completely methylated manninositose, and obtained an equimolecular mixture of tri- and tetra-*O*-methylmannoses, while periodate oxidation showed the 1→6 bonding of the two mannose residues. On the basis of these results the structure (XV) was assigned to manninositose. This structure was even confirmed by synthesis[36], but in 1964 Lee and Ballou[37], in a brilliant paper on the myoinositol mannosides from the glycolipids of *Mycobacterium tuberculosis* and *Mycobacterium phlei*, showed it to be untenable, and established the structure of manninositose (XVI) and the pentamannoside of phosphatidylmyoinositol.



Exhaustive periodate oxidation of dimannosylmyoinositol with subsequent reduction of the polyaldehydes with sodium tetrahydroborate and acid hydrolysis of the reduction products yielded arabitol (XVII) (the D-configuration was established for (XVII) in the case of the pentamannoside of

myoinositol) and glycerol (from carbon atoms 4, 5, and 6 of the mannoses) in the proportions 1 : 2:

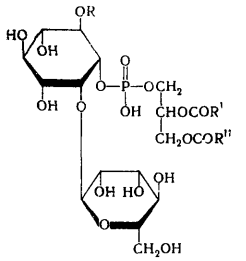


(XVII)

Exhaustive methylation of the dimannoside of myoinositol followed by methanolysis led to the formation of two moles of 2, 3, 4, 6-tetra-*O*-methylmannose per mole of 1, 3, 4, 5-tetra-*O*-methylmyoinositol. The structure of the latter was confirmed also by the nuclear magnetic resonance spectra. The α-glycosidic bonds in the dimannoside of myoinositol were established from the signal of the anomeric proton in the n.m.r. spectrum of this compound.

The phosphatidic acid residue in mannosides of phosphatidylmyoinositol should be present in position 1 of the myoinositol ring, since only this can explain the formation on periodate oxidation from the same products as in the oxidation of myoinositol dimannoside. Thus the glycolipid from *Mycobacterium tuberculosis* is 1-phosphatidyl-2, 6-di-*O*-α-D-mannopyranosyl-(1*S*)-myoinositol (XVIII)[37]. The presence of mannose residues on both sides of the phosphate–myoinositol bond explains the stability of the phosphate diester bridge to alkaline hydrolysis and the unusual stability of intact glycolipids in hot acetic acid (absence of free hydroxyls on the myoinositol ring carbon atoms adjacent to the phosphate bond).

The structure of the myoinositol monamannoside isolated from *Mycobacterium tuberculosis* was proved by exhaustive methylation followed by methanolysis and identification of the resulting pentamethylmyoinositol by comparison with all four possible isomers in gas–liquid chromatography[38]. It was established that the mannose was bound to the 2-hydroxyl of the myoinositol, i.e. that the intact lipid has the structure (XIX):

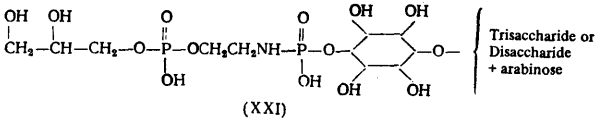


(XVIII: R = α-D-mannopyranosyl); (XIX: R = H); (XX: R = α-D-mannopyranosyl-(1 → 2)-*O*-α-D-mannopyranosyl-(1 → 6)-*O*-α-D-mannopyranosyl-(1 → 6)-*O*-α-D-mannopyranosyl).

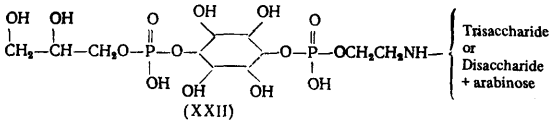
The biosynthesis of phosphatidylmyoinositol dimannoside is probably accomplished by the stepwise glycosylation of phosphatidylmyoinositol, firstly at position 2 and then at position 6 [39].

Complete structures were later established also for the tri-, tetra-, and hexa-mannosides of phosphatidylmyoinositol[40]. Thus the pentamannoside was proved to have the structure 1-phosphatidyl-2-*O*-α-D-mannopyranosyl-6-[*O*-α-D-mannopyranosyl-(1 → 2)-*O*-α-D-mannopyranosyl-(1 → 6)-*O*-α-D-mannopyranosyl-(1 → 6)-*O*-α-D-mannopyranosyl)]-(1*S*)-myoinositol (XX). Pangborn and McKinney[41] have recently shown that, in the serologically active phosphoinositides of *Mycobacterium tuberculosis*, the ratio of fatty acids to phosphorus is 3 : 1 or 4 : 1; palmitic and tuberculostearic acids were found most frequently among the fatty acids, while little stearic and oleic acids were found. The authors assume that the "excess" acids can be esterified by any free hydroxyls (one or two) in the myoinositol or mannose residues. The same conclusion was reached by Brennan and Ballou[42], who studied the biosynthesis of several mannosylphosphatidylmyoinositols catalysed by intracellular fractions of *Mycobacterium phlei*. They included the three substances which they obtained among the dimannophosphoinositides. The ratio of fatty acids to phosphorus in these compounds was 4 : 1, 3 : 1, and 2 : 1.

Great interest attaches to establishing the structure of the intact complicated lipid complexes which are assumed to be present in animal and plant organisms. One of the first attempts to penetrate the structure of the complicated "inositide complex" was the hypothesis of approximate structures of the inositol phosphatide from groundnuts (XXI and XXII)[6,20]:

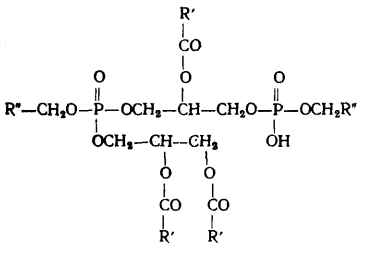


(XXI)

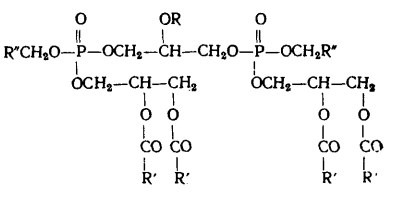


(XXII)

Several authors consider that natural lipid complexes are triesters of phosphoric acid. This view was first expressed by Collins[43], who suggested the possible structures (XXIII) and (XXIV) for such complexes (R = H). Unfortunately, only a few papers have yet been published on the study and structure of lipid "triester complexes". Sinha and Gaby[44] investigated the lipo-amino-acid complexes from *P. aeruginosa*, and regarded these compounds as triesters of phosphoric acid having the structure (XXIV), with R = OCCH(NH$_2$)R‴, the R′ being residues of fatty acids, and R″ residues of nitrogen bases:



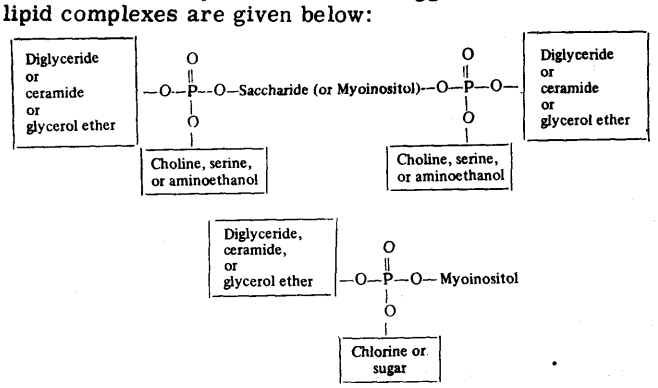
(XXIII)



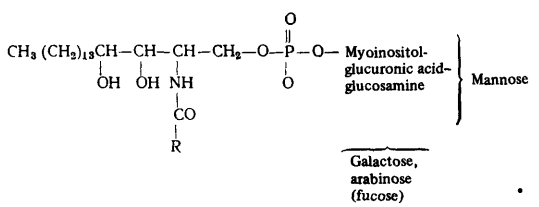
(XXIV)

As already remarked, Galanos and Kapoulas[4,22,23] developed a technique for isolating lipid complexes from natural sources, and proposed several structures for such complexes, in which myoinositol is an important component. They consider that "triester complexes" are actually present in animal and vegetable tissues, while individual phospholipids are merely products of their degradation during isolation from natural sources. These authors note especially the role of myoinositol in stabilising

the "triester complexes". Some suggested structures for lipid complexes are given below:



A series of papers was published by Carter [45] on the isolation from plant seeds of phytoglycolipids containing myoinositol. A study of the degradation products of the phytoglycolipid led to the proposed structure [46]



In 1966 as phingolipid containing dihydrosphingosine, myoinositol, and mannose was isolated from the microorganisms *Torulopsis utilus* [47].

Further work on the isolation, structure, and synthesis of complicated triester lipid complexes should finally settle the question of their presence in animal and vegetable tissues and their structure.

III. BIOCHEMISTRY OF INOSITOL PHOSPHATIDES

The biochemistry of inositol phosphatides, as members of the class of phospholipids, has been a subject for research in many laboratories, and is quite widely represented in individual papers, reviews, and monographs (e.g. Hawthorne's review [48]). The present Section will survey work done in recent years on properties and functions characteristic of inositol phosphatides.

Phosphoinositides, together with other phospholipids, form structural components of cells. An increased content of inositol phosphatides is found in the cells of certain tissues. Thus the percentage content of inositol phosphatides (and sphingomyelin) in the cells of the lens of the human eye is considerably higher than in other tissues of the body, where lecithin predominates in the phospholipid fraction [49]. It has been suggested that inositol phosphatides have some specific function as structural components of cell membranes in the filaments of the lens of the human eye. Myoinositol labelled with ^{14}C is actively incorporated into the cytoplasmic membranes of the fungus *Neurospora crassa* [50], where it was found that cytoplasmic membranes containing protease were relatively impoverished in lecithin and enriched in inositol phosphatides. Structural integration of protease particles was disturbed in mutants of *N. crassa* grown on a medium containing an insufficient quantity of myoinositol.

Much work has recently been done on the properties of monomolecular films of various phospholipids, which in some measure simulate biological membranes. Studies have been made of the hydrolysis by phospholipase A of monolayers [51] of lecithin, phosphatidyl-aminoethanol, and phosphatidylinositol all labelled with ^{32}P. The change in the surface potential of the film served as a measure of the degree of decomposition of the lipid. Loss of radioactivity by the film on hydrolysis was due to escape of the lyso-compound of the film. Physicochemical and biochemical study of enzymatic hydrolysis in the presence of calcium ions showed the lowest rate of hydrolysis to occur in films of ^{32}P-phosphatidylinositol, which possesses the highest affinity for Ca^{2+}.

Several workers have isolated enzymes specific to inositol phosphatides. Hawthorne [52] isolated from the guinea-pig a phosphoinositideinositolphosphohydrolase which acted on mono-, di-, and tri-phosphoinositides without hydrolysing other phospholipids. Enzymatic hydrolysis yields a diglyceride and inositol phosphates; no inorganic phosphate was detected. English et al. [53] isolated an enzyme termed myoinositolkinase. Myoinositol 1-phosphate was detected among the reaction products.

Inositol phosphatides take part in such important processes in the cell as the change in size of the mitochondria and active transport through the membrane [54]. A "contractile" protein, which was responsible for contraction of the mitochondria on the addition of adenosine triphosphate and magnesium ions, lost its activity on extraction with solvents which remove lipids. The active component was a phosphatidylinositol at a concentration of 10^{-6} M. Other lipids do not possess such activity [55]. In a study of the metabolism of the phosphoinositide complex in the salivary gland of the cat on electrical stimulation, Gaut et al. [56] concluded that increased metabolic activity occurred in the phosphoinositide complex, and they suggested that a phosphoinositide combined with a membrane protein may play an active part in transport processes in the cell. Lipo-amino-acids, in which considerable interest has recently been shown, may also be involved in active transport through the membrane. It is suggested [57], for example, that the synthesis of antibodies involves the formation of a lipo-amino-acid complex on or in the cell membrane, by means of which the amino-acid enters the cell. These complexes may, perhaps, take part in protein synthesis. They may well have the structure of triesters of phosphoric acid [23]. Phosphatidylinositol has been detected in the lipid portion of phosphatidopeptides from lamellae and chloroplasts of the bean. The stability of phosphatidopeptides to acid hydrolysis and their infrared spectra indicate a lipid–peptide ester link [3].

An active part in transport through the membrane is suggested also for the polyphosphoinositides found in various organs and tissues. The metabolism of the phosphoinositide complex of brain was first investigated by Brockerhoff and Ballou [39,58], who showed the path of biosynthesis from monophosphoinositide by phosphorylation to di- and tri-phosphoinositides. Adenosine triphosphate may be the donor of the phosphate group. Later work [59] has confirmed that the incorporation of ^{32}P-orthophosphate into the diphosphoinositide of mitochondrial fractions from the liver is diminished by inhibitors of oxidative phosphorylation. Dephosphorylation takes place together with phosphorylation in the inositide complex, so that a metabolic process is occuccring:

$$\text{Monophosphoinositide} \rightleftharpoons \text{diphosphoinositide} \rightleftharpoons \text{triphosphoinositide}$$

Perhaps this may also explain the biochemical role of the inositide complex in the transport of cations [60].

The extraordinarily high metabolic activity of the inositide complex and its participation in active transport through the membrane have been discussed in many papers during the past three years. Hawthorne[61], studying the place of synthesis of the diphosphoinositides of rat liver, found that they were synthesised mainly in plasmic membranes. He even demonstrated the synthesis of polyphosphoinositides *in vitro* catalysed by a homogenate of rat brain[62]. The high metabolic activity of phosphoinositide complexes is explained by the presence of polyphosphoinositides in them. Freyss et al[63] showed a high rate of metabolism of inositol phosphatides compared with other phospholipids, when studying the metabolism of various phospholipids in the brain of the rat, and Nicolas[64] reached the same conclusion, having established a more rapid loss of radioactivity by the inositol phosphatides of myelin labelled with 1-^{14}C-acetate, compared with cerebrosides, phosphatidyl-aminoethanol, and other lipids.

The biochemistry of other inositol phosphatides is also unusual. Inositol monophosphatides containing residues of two different fatty acids, isolated from the lipid fractions of horse brain, are found[65] to be blood anticoagulants. Bacterial glycolipids possess serological activity. Phosphatide antigens of several mycobacteria have been identified as mannosides of phosphatidylinositol[66]. They fix complement in a serum containing antibodies against tubercle bacilli. The glycolipids of acid-stable bacteria are very toxic: an adult mouse is killed 5–8 days after the injection of 5–10 μg. The cause of death is not yet clear. The injection of certain lipid fractions of tubercle bacilli produces immunisation of animals, increasing, for example, the lifetime of a mouse which has been inoculated with virulent tubercle bacilli. Detailed information on the biological role and functions of the glycolipids of acid-resistant bacteria will be found in Lederer's review[67].

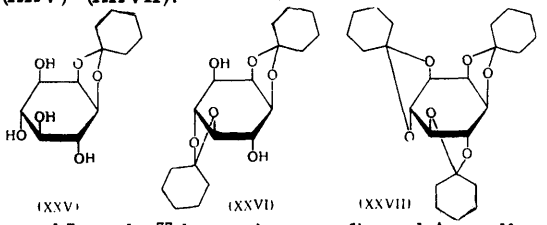
Many papers have been published also on the biosynthesis and the metabolism of myoinositol[68-70]. It has been established that myoinositol plays a part in cellular morphogenesis, lipid synthesis and transport, the transport of ions and hormones through cell membranes, and other important vital processes[71]. A detailed account of the biochemistry of myoinositol has been given in a recent review by Posternak[72].

IV. SYNTHESIS OF INOSITOL PHOSPHATIDES

Besides a biochemical study, many laboratories are occupied with synthetic investigations on the inositol phosphatides. Phosphoinositides themselves—in particular their main component, myoinositol—provide a wide field of activity for organic chemists. The urgent requirements of biochemistry and biology also lead to synthetic investigations on phospholipids. For example, Collins[43] regards the synthesis of triesters of phosphoric acid, the structure which he suggested, as having great value in confirming their presence in Nature and in studying their properties. In fact, the isolation of such labile substances from natural sources requires colossal amounts of labour, and ultimately does not yield quantities sufficient for a systematic study of their properties and biological activity. A similar opinion is held by Ballou[37,38].

A comprehensive study of the chemistry of myoinositol is being made in Angyal's laboratory. This work is of great value for the synthesis of inositol phosphatides. The myoinositol molecule (I) contains six hydroxyls, five of which are equatorial, while one, in position 2, is axial. The myoinositol molecules have the chair form, as has been confirmed by X-ray examination[73]. This structure creates fundamental difficulties in the synthesis of inositol phosphatides in which myoinositol is combined with phosphate in position 1. Various protective groups for the hydroxyls have been studied, but until recently none of them has enabled a protected myoinositol to be prepared with a free 1-hydroxyl, a key intermediate in the synthesis of inositol phosphatides. Isopropylidene protection was used in the early work, and with its help methyl ethers of myoinositol were synthesised in low yields[74].

Subsequently cyclohexylidene derivatives of myoinositol, prepared in good yield by reaction with cyclohexanone, have come to be employed. Angyal[75,76] has prepared mono-, di-, and tri-cyclohexylidene derivatives of myoinositol (XXV)–(XXVII):

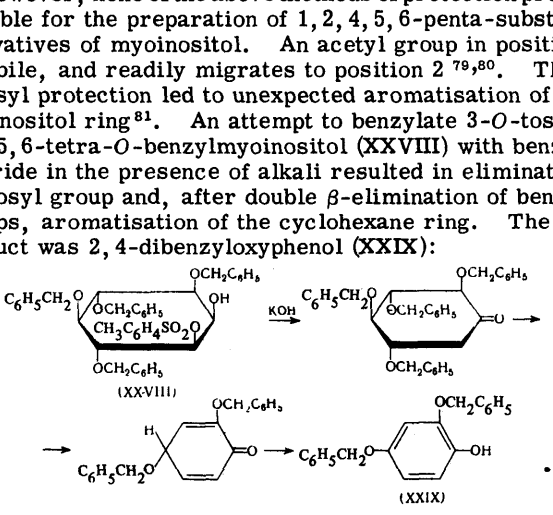


(XXV) (XXVI) (XXVII)

Mager and Ionesku[77] have since confirmed Angyal's observation that acetals are first formed at *cis*-hydroxyls (*ae*), and only then at *trans*-hydroxyls (*ee*). Angyal and Tate[78] have synthesised myoinositol 4- and 5-monophosphates and 1,4-, and 1,6-, and 4,5-diphosphates from cyclohexylidene derivatives of myoinositol.

Further efforts were directed at the selective protection of the 2-hydroxy-group in 1,4,5,6-tetra-substituted derivatives of myoinositol, which are easily obtained from 1,2-cyclohexylidenemyoinositol (XXV). It was shown that several substituents occupy the 3-equatorial position preferentially, whereas others do not display such selectivity. Thus the acetylation[79], tosylation[80], benzylation, and methylation[81] of the tetra-substituted derivatives take place selectively at position 3, whereas tetrahydropyranyl protection is not selective[82]. It was suggested[81] that reactions involving a transition state (etherification, substitution by an S_N2 mechanism) take place preferentially at position 3 owing to great steric hindrance at position 2. Rapid reactions (substitution by an S_N1 mechanism) are less sensitive to steric hindrance, and therefore occur non-selectively.

However, none of the above methods of protection proved suitable for the preparation of 1,2,4,5,6-penta-substituted derivatives of myoinositol. An acetyl group in position 3 is labile, and readily migrates to position 2 [79,80]. The use of tosyl protection led to unexpected aromatisation of the myoinositol ring[81]. An attempt to benzylate 3-*O*-tosyl-1,4,5,6-tetra-*O*-benzylmyoinositol (XXVIII) with benzyl chloride in the presence of alkali resulted in elimination of the tosyl group and, after double β-elimination of benzyl groups, aromatisation of the cyclohexane ring. The final product was 2,4-dibenzyloxyphenol (XXIX):

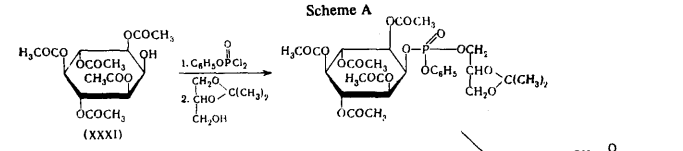


(XXVIII) (XXIX)

In 1966 the preparation was reported of DL-1,2,4,5,6-penta-*O*-benzylmyoinositol[83], which serves as a convenient intermediate for the synthesis of racemic inositol phosphatides. In this synthesis Gigg used allyl protection, previously employed in the chemistry of carbohydrates[84]. Allyl protection was introduced selectively in position 3 of 1,4,5,6-tetra-*O*-benzylmyoinositol, the product was then benzylated at position 2, and after isomerisation of the allyl group into propenyl the latter was removed by acid hydrolysis. Finally, Angyal[85] has recently shown that 1-benzyl-2,3,4,5,6-penta-acetylmyoinositol can be obtained by the acetolysis of 1,4,5,6-tetrabenzylmyoinositol, owing to steric hindrance to acetolysis of the benzyl group at position 1. Hydrogenolysis of the product yielded 1,2,4,5,6-penta-acetylmyoinositol. All this work shows how complicated and interesting is the problem of the selective protection of the hydroxy-groups in myoinositol.

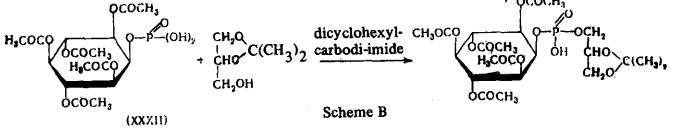
The first synthesis of a product of the hydrolysis of inositol phosphatides—myoinositol 2-monophosphate—was accomplished in 1949 by Iselin[86], by the reduction of scyllo-mesoinosose penta-acetate with subsequent phosphorylation with diphenyl phosphorochloridate and removal of the protective groups. As already remarked in Section II, it was thought at that time that the phosphate in inositol phosphatides was attached to the $C_{(2)}$ atom of the myoinositol molecule. Only after ten years did Davies and Malkin[87] and at the same time Ellis and Hawthorne[88] synthesise 1-glyceryl-2-myoinositol phosphate (XXX) by different methods: the former by the reaction of 1,3,4,5,6-penta-*O*-acetylmyoinositol (XXXI) with phenyl phosphorodichloridate and 1,2-isopropylideneglycerol (Scheme A); and the latter by condensing (XXXII), the 2-phosphate of (XXXI), with 1,2-isopropylideneglycerol in the presence of dicyclohexylcarbodi-imide (Scheme B).

Scheme A



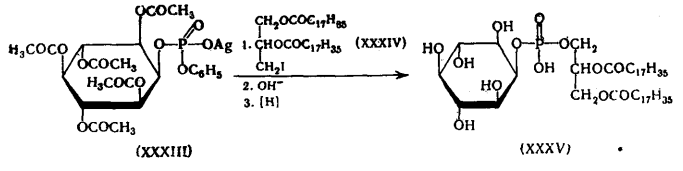
(XXXI) (XXX)

Scheme B



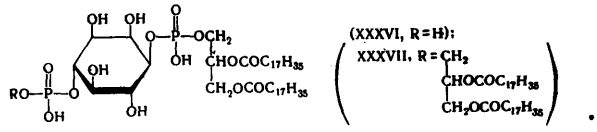
(XXXII)

Davies and Malkin[89] also obtained the inositol phosphatide (XXXV) by condensing the silver salt of a protected 2-phosphate of myoinositol (XXXIII) with the iodide of 1,2-distearoylglycerol (XXXIV), but it subsequently became clear that the product did not have the natural structure:



(XXXIII) (XXXIV) (XXXV)

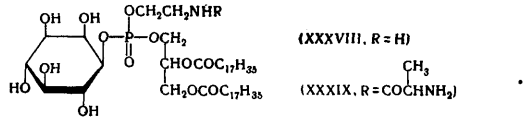
Several complicated inositol phosphatides of natural structure have been synthesised during recent years in Preobrazhenskii's laboratory. Thus Preobrazhenskii, Shvets, et al.[90] prepared the 1,4-diphosphoinositides (XXXVI) and (XXXVII) by condensing 1,2:4,5-dicyclohexylidenemyoinositol (XXVI) with phosphoryl chloride and 1,2-distearoylglycerol:



(XXXVI, R=H); (XXXVII, R=CH_2)

These compounds may be fragments of more complicated lipid complexes.

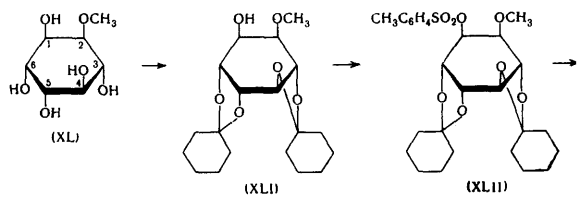
The same workers[91] were the first to obtain inositol phosphatides having the structure of triesters of phosphoric acid (XXXVIII), and simulating a possible bond between the lipid and the amino-acid moieties in lipo-amino-compounds (XXXIX):



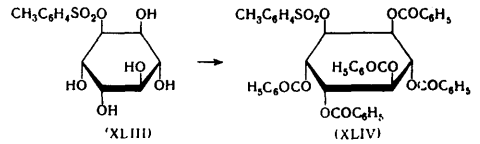
(XXXVIII, R=H) (XXXIX, R=$COCHNH_2$)

Unfortunately, all the inositol phosphatides described were isolated in the optically inactive, racemic form. In natural phosphoinositides the myoinositol molecule is asymmetrically substituted in the equatorial 1-position, and hence possesses optical activity. This has been demonstrated both for simple inositol phosphatides[26-30] and for the complicated complexes. It has been established[92], for example, that brain triphosphoinositide has the structure 2,3-di-*O*-acylglyceryl-1-[(1′*S*)-myoinositol 1′,4′,5′-triphosphate].

In practice the problem of synthesising optically active inositol phosphatides has still not been solved. It is true that Ballou[29] reported in 1959 the synthesis of optically active myoinositol 1-phosphate, which can serve as intermediate for the synthesis of optically active inositol phosphatides. The starting material in this synthesis was galactin (II), which was completely benzylated by Kuhn's method; methanolysis with 1% hydrochloric acid led to cleavage of the α-glycosidic bond in nonabenzylgalactin, the resulting 1,2,4,5,6-penta-*O*-benzylmyoinositol was phosphorylated with diphenyl phosphorochloridate in the free 3-position, and after removal of protective groups the optically active myoinositol 1-phosphate was obtained[93]. Gero[94] has recently suggested a different route to this optically active compound, starting from quebrachitol, 2-*O*-methyl-(−)-inositol (XL). 3,4:5,6-Di-*O*-cyclohexylidenequebrachitol (XLI) was obtained[73] from (XL), and then converted into 3,4:5,6-di-*O*-cyclohexylidene-2-*O*-methyl-1-*O*-tosyl-(−)-inositol (XLII). After elimination of the methyl group with boron trichloride in dichloromethane at −80°C the unstable 1-*O*-(−)-inositol (XLIII) was obtained. This was benzoylated, and optically active myoinositol 1-phosphate could be obtained from the final products, 2,3,4,5,6-penta-*O*-benzoyl-1-*O*-tosyl-(−)-inositol (XLIV), after removal of the tosyl group, inversion of the 1-hydroxyl, and phosphorylation:

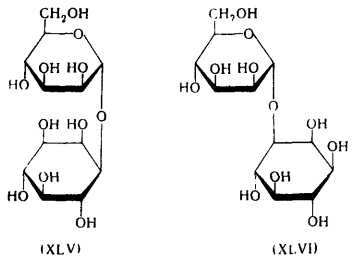


(XL) (XLI) (XLII)



(XLIII) (XLIV)

However, optically active naturally occurring compounds—galactin and quebrachitol—were used as starting materials in these syntheses[93,94]. Purely chemical methods of obtaining optically active 1-*O*-substituted myoinositols have not yet been developed because of the great difficulty of separating the resulting racemic mixtures into optical isomers. The only example of such a separation is the work of Angyal and Shelton[95] on the synthesis of 1-*O*-α-D-mannopyranosylmyoinositols—(XLV) and (XLVI)—and α-D-mannopyranosyl-(1→6)-*O*-α-D-mannopyranosyl-(1→1)-myoinositol (XV). Mannosylmyoinositol was obtained by the Königs-Knorr reaction from 1,4,5,6-tetra-*O*-acetylmyoinositol and tri-*O*-acetyl-α-D-mannopyranosyl bromide, the low yield (4.5%) being explained by large steric hindrance. Mannosylmyoinositol is easily separated into two diastereomers—1*S* (XLV) and 1*R* (XLVI)



(XLV) (XLVI)

These diastereomers could have served as starting material for the preparation of optically active 1-*O*-substituted derivatives of myoinositol, but the low yield gives no grounds for regarding this work as a practical route to optically active phosphoinositides. The same workers[95] synthesised galactin in 2.1% yield by the Königs-Knorr reaction. In this case the diastereomers were separated by seeding with crystals of natural galactin.

During recent years great successes have been achieved in studying the chemistry and the biochemistry of inositol phosphatides. The structure of several "inositide complexes" has been elucidated, and methods have been developed for isolating them from vegetable and animal tissues. Inositol phosphatides undoubtedly play an important part in the most intimate processes in the cell. But the structure of many complicated phosphoinositides, their complexes with amino-acids and proteins, and the biological role of such compounds have hitherto formed subjects of hypothetical assumptions. Although there are considerable achievements in the chemistry of inositol phosphatides, in the synthesis of complicated lipid complexes simulating possible natural structures, the problem of preparing optically active phosphoinositides, which are of the greatest interest for biology, is still unsolved. The synthesis of these compounds will probably be accomplished in the near future.

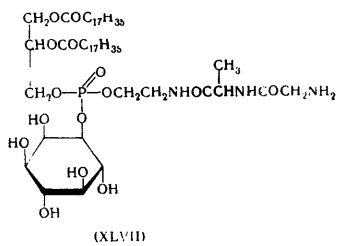
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Many papers on the structure, biochemistry, and synthesis of inositol phosphatides have been published while the manuscript was being prepared for press.

Scamark et al.[96] have shown for the first time that 1-*O*-(D-glycerylphosphoryl)-(1*S*)-myoinositol is present as a normal component in biological tissues, not as a degradation of complicated phosphoinositides. They isolated this compound from the prostate gland and the epididymis of rats, and from bull and ram semen. Di- and tri-phosphoinositides have been isolated from baking yeast (*Saccharomyces cerevisiae*)[97].

The detection of mannophosphoinositides in various species of bacteria indicates the close phylogenetic and taxonomic relations of these species. Mannophosphoinositides have been isolated from *Propionibacterium shermanii*[98], *Streptomyces sioyaensis*[99], *Corynebacterium xerosis*[100], and others[101,102]. Ballou and Brennan[103] have continued to study the biosynthesis of mannophospjoinositides of *Mycobacterium phlei*. The enzymatic acylation of dimannophosphoinositides is discussed in the same paper. The high metabolic activity of phosphoinositides is confirmed in several papers. A tracer is incorporated into the phosphatidylinositol in *Nocardia polychromogenes* eight times as rapidly as into a cardiolipin[104]. Nakatani et al.[105] have investigated the incorporation of inorganic phosphate *in vitro*. Phosphorus-32 is incorporated more rapidly into phosphatidylinositol than into lecithin (in the coronary artery of the bull, for example, by factors of 4.9–5.3). It has been shown[106] that acetylcholine stimulates cleavage of the phosphodiester bond in the phosphoinositides of the guinea-pig brain. Enzymes specific to phosphoinositides continue to be studied. Thus Lee and Huggins have reported on the structure[107], purification, and properties[108] of triphosphoinositidephosphomonoesterases from rat kidneys. The role of phosphoinositides and complicated inositide complexes in organising the membranes of the nervous system is discussed from the point of view of evolution in Kreps's book[109].

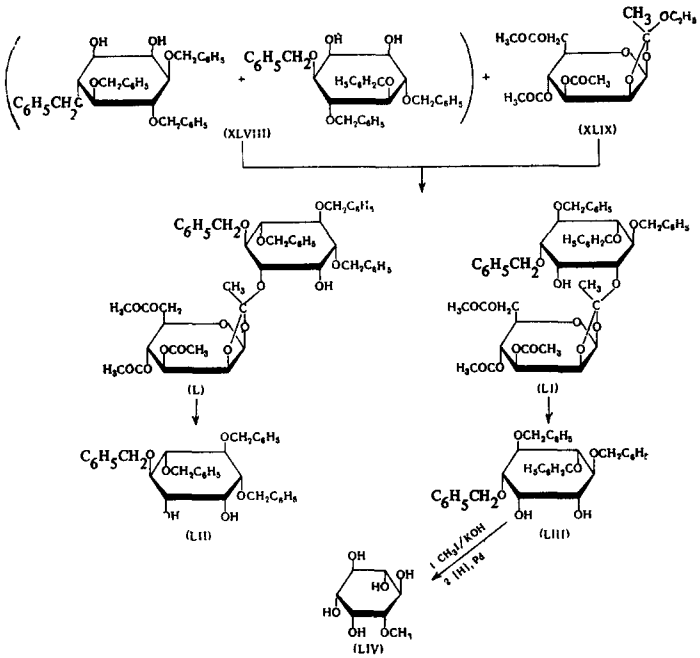
N. A. Preobrazhenskii's school enjoys a leading position in synthetic investigations on the inositol phosphatides. In 1968 Preobrazhenskii, Zhdanovich, Lyutik, et. al.[110] synthesised *O*-[α-(α′β-distearoyl)glycerylphosphoryl-(1-myoinositol)]-*N*′-(glycylalanyl)amino-ethanol (XLVII), the simplest phosphatidopeptide, which is a model of possible structures for the phosphatidopeptide fractions from tissues of brain, liver, etc. The same workers[111] have reported the synthesis of a monophosphoinositide containing the phosphatidyl residue in position 1, the starting material being 1,2,4,5,6-penta-*O*-acetylmyoinositol[85]. Preobrazhenskii, Tarusova, Kozlova, et al. have investigated[112] the benzoylation of 1,2:4,5-dicyclohexylidenemyoinositol (XXVI), and have shown that position 3 in the myoinositol is benzoylated preferentially.



(XLVII)

Continuing his previous work[94] aimed at obtaining optically active phosphoinositides, Géro[113] has synthesised 1,2,4,5,6-penta-*O*-benzoyl-(1*S*)-myoinositol, using quebrachitol as starting material. Unfortunately, the optical isomer which he has obtained does not yield phosphoinositides of natural structure, in which the phosphatidyl residue is bound[26-30] to the (1*S*)-position in myoinositol.

Preobrazhenskii and the present Reviewers [114] have recently developed a method for separating racemic asymmetrically substituted myoinositols via diastereomeric orthoesters with carbohydrates, which enables both optically active isomers to be isolated in optically pure form. 1,4,5,6-Tetra-*O*-benzylmyoinositol (XLVIII) was subjected to transesterification with 3,4,6-tri-*O*-acetyl-1,2-*O*-ethylorthoacetyl-β-D-mannopyranose (XLIX), the resulting diastereomeric orthoesters (L) and (LI) were separated by crystallisation and chromatography, and 1,4,5,6-tetra-*O*-benzyl-(1*S*)-myoinositol (LII) and its (1*R*)-enantiomer (LIII) were isolated from the separated orthoesters (L) and (LI) by mild acid hydrolysis. The first complete synthesis of (−)-bornesitol (LIV) has been accomplished from the (1*R*)-enantiomer.



The possibility of separating various asymmetrically substituted myoinositols into enatiomers opens up a path to the synthesis of optically active phosphoinositides of natural structure.

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