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From the Trioses to the Synthesis of Natural Phospholipids: A Research Trail of Forty Years

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I WOULD LIKE TO EXPRESS my sincerest thanks to the American Oil Chemists' Society and to Dr. Arthur Rose of the Applied Science Laboratories, for the signal honour bestowed upon me in selecting me as the first recipient of the newly established award in lipid chemistry. At the same time I would like to take the opportunity to acknowledge publicly my great debt to past and present colleagues whose exceptional skills and untiring efforts have contributed so greatly to fashioning the methods for the synthesis of some of nature's most intriguing biological compounds. I consider the award as a tribute to the successes that have crowned the efforts of our team.

One of the privileges enjoyed by the recipient of an award is the opportunity to present, to a discerning audience, a review of some aspects of his work. When the letter of invitation to participate at this symposium suggested that I speak on the synthesis of phospholipids, I accepted with pleasure as this has been my major interest for many years. Many others have contributed to this field of course, but today for obvious reasons I shall restrict myself to the work carried out in my laboratory. The main theme of my lecture will be the chemical synthesis of natural phospholipids, and the elucidation of their structure and configuration.

You may wonder perhaps, how I became interested in the synthesis of phospholipids. My scientific career began about 40 years ago, with a study of the chemical properties of carbohydrates containing only three carbon atoms. I did not realize then that these studies ultimately would lead me into a branch of biochemistry which encompasses groups of compounds whose significance to life processes has become in recent years the subject of intensive research. My doctoral thesis, the work which I carried out from 1925–1927 under the guidance of my "Doktor-Vater" and later good friend, Hermann O. L. Fischer, de-

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scribed the isomerization of glyceraldehyde to dihydroxyacetone (1,2). This rearrangement reaction was an accidental discovery, and was made while trying to obtain the monomeric form of glyceraldehyde by heating a suspension of its crystalline, dimeric form in pyridine to reflux temperature. The chemistry of the trioses, which even today is somewhat neglected by most carbohydrate chemists, was the subject of my research for many years. Those studies provided the theoretical foundations as well as the starting materials for the synthesis of optically-active glycerol derivatives. One of the many derivatives of the trioses which I prepared in Dr. Fischer's laboratory at that time, and which was to become widely known, was DL-glyceraldehyde-3-phosphate (3). This compound, which later came to be referred to as Fischer-Baer ester, achieved some prominence because of its central role in carbohydrate metabolism. Its synthesis in 1932 revealed the chemically labile nature of the triose-phosphates, and had considerable stimulatory effect on the development of the Embden-Meyerhof Scheme for glycolysis and fermentation. From the synthesis of glyceraldehyde-3-phosphate it was quite natural to proceed to the synthesis of α-glycerolphosphoric acid (4–6), which chemically as well as biochemically is closely related to the triose-phosphates and is a moiety of many phospholipids. Thus it was almost inevitable that finally I should become interested in the synthesis of the more complex esters of glycerolphosphoric acid, i.e. the glycerolphospholipids.

It is well known to most of you that phospholipids generally occur in nature as mixtures of great complexity, and that their separation offers unusual difficulties. The isolation of pure, individual phospholipids from natural sources has been accomplished in very few instances, and only when aided by particularly favourable circumstances. Although most observations by earlier workers were made on mixtures of phospholipids, they were nevertheless sufficient to postulate provisional structures for some of these compounds. There remained, however, the task of establishing with certainty both the position of the phosphate ester group in the glycerol molecule, and the stereochemical classification of the phospholipids.

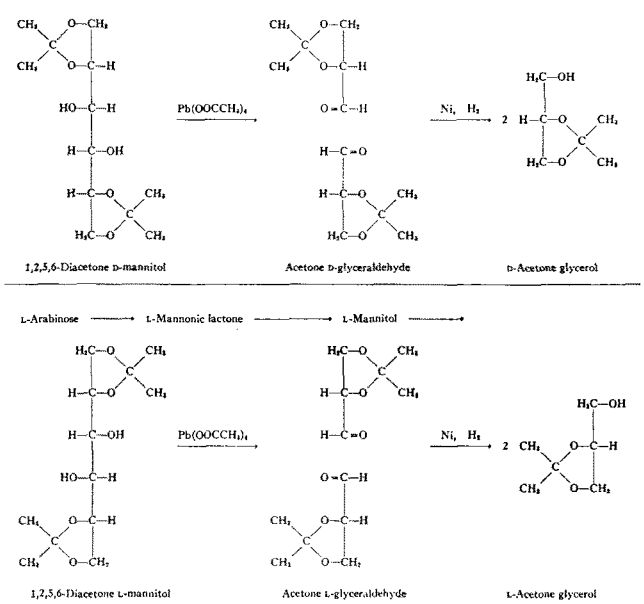


FIG. 1. Synthesis of D- and L-acetone glycerol. Fischer and Baer (1937, 1939).

The best-known group of glycerolphospholipids, and apparently the one occurring most widely in nature, comprises the lecithins. The discovery of lecithin was made by Gobley, who found it to be present in egg yolk, in the eggs and milt of carp, in the brain of hens, and in the blood of man and ox. The structure of lecithin suggested by Strecker is the one which is now generally accepted. In it, the phosphoric acid is attached to one of the primary hydroxyl groups of glycerol. This formula was strongly supported by the observation of Willstätter and Lüdecke (7) that the glycerolphosphoric acid they had obtained on hydrolysis of lecithin was optically active, although the rotation was very small. The glycerolphosphoric acid can be optically active only if the phosphoric acid is attached to one of the terminal (α) hydroxyls of glycerol. Later investigations of Karrer and Salomon (8) revealed that the glycerolphosphoric acid obtained by either hydrolysis or saponification of lecithin consists of a mixture of α- and β-glycerolphosphoric acid. The presence of β-glycerolphosphoric acid in the hydrolysates of lecithin was interpreted as an indication of the existence in nature of β-lecithins. All attempts, however, to isolate a β-lecithin from natural sources without success.

A resolution of the unsatisfactory state of knowledge regarding the exact structure of natural glycerolphospholipids became possible when Dr. Fischer and I succeeded in the late thirties in synthesizing the optically active forms of acetone glycerol, i.e., D-acetone glycerol (9,10) and L-acetone glycerol (11). *These asymmetrically substituted and optically active glycerol derivatives not only were to become the key substances in the synthesis of optically active glycerides and phospholipids, but by virtue of their known stereochemical relationship to* D- *and* L-*glyceraldehyde made it possible to establish by unambiguous methods the configuration of naturally occurring glycerides and glycerolphospholipids.*

Since D- and L-acetone glycerol are the starting materials for all of our syntheses in the phospholipid field, I would like to show you how these substances are obtained (Reaction Fig. 1): D- and L-mannitol, respectively, are acetonated by means of zinc chloride and acetone, and the resulting 1,2–5,6-diacetone mannitols are oxidatively cleaved with lead tetraacetate. The cleavage products, i.e. 2 moles of acetone D-glyceraldehyde or 2 moles of acetone L-glyceraldehyde on catalytic reduction yield D-acetone glycerol and L-acetone glycerol. The synthesis of these two compounds was crucial for the development of procedures for the synthesis of enantiomeric glycerides and phospholipids. All procedures developed more recently by other workers in this field depend entirely,

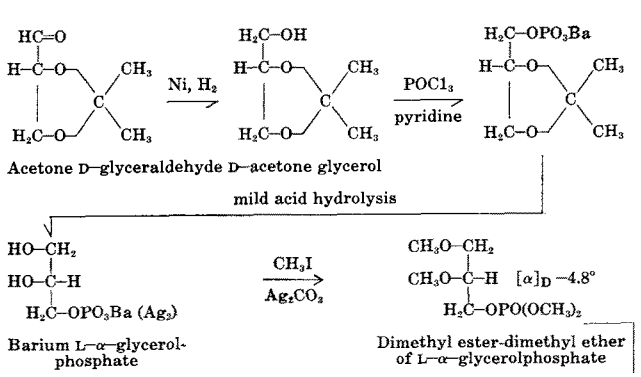


FIG. 2. Synthesis of L-α-glycerolphosphate. Fischer and Baer (1937).

as do our own, on D- and L-acetone glycerol for the preparation of optically active phospholipids.

The phosphorylation of D-acetone glycerol with phosphorus oxychloride and quinoline, and removal of the protective acetone group by mild acid hydrolysis (Fig. 2) gave structurally pure α-glycerolphosphoric acid with a specific rotation of −1.45° in 2N hydrochloric acid (5). This is a very small rotation. It accounts for the difficulties experienced by earlier investigators in deciding whether or not the small rotation of glycerolphosphoric acid obtained from natural lecithins by hydrolysis is a property of this substance or is caused by small amounts of highly active impurities. Exhaustive methylation of the silver salt of the weakly optically active α-glycerolphosphoric acid with methyl iodide and silver carbonate gave the dimethyl ether of dimethyl α-glycerolphosphate with the considerably higher specific rotation of −4.8°.

Having succeeded in preparing one of the two stereoisomers of α-glycerolphosphoric acid, its configuration had to be determined. How this was accomplished is shown by Figure 3. The lower series of reactions illustrates again the synthesis of α-glycerolphosphoric acid from D-acetone glycerol. As can be seen its phosphoric acid is attached to the hydroxyl group which is formed by reduction of the carbonyl group of acetone D-glyceraldehyde. It is thus in the opposite position to that occupied by the phosphoric acid of D-glyceraldehyde-3-phosphate or its reduction product, D-α-glycerolphosphate. The phosphorylation of D-acetone glycerol thus yields L-α-glycerolphosphoric acid. By comparing the optical activities of the dimethyl-ester, dimethyl-ethers of synthetic L-α-glycerolphosphoric acid and of glycerolphosphoric acid obtained by hydrolysis of natural lecithins, we were able to prove conclusively that the hydrolysis products of natural lecithins contain L-α-glycerolphosphoric acid. Since, it can only be the hydrolysis product of lecithins containing L-α-glycerolphosphoric acid as moiety, the natural occurrence of L-α-lecithins became fairly certain. We hesitated, however, to accept the presence of β-glycerolphosphoric acid in the hydrolysis products of lecithins as a valid proof for the natural existence of β-lecithins. Our reluctance was supported by the fact that Bailly (12), Chargaff (13) and Verkade et al. (14) had shown that α-glycerolphosphoric acid in hot acid solution undergoes an intramolecular rearrangement with the formation of an equilibrium mixture of α- and β-glycerolphosphoric acid. We strongly suspected that the presence of β-glycerolphosphoric acid in the hydrolysis products of natural lecithins is the result of a similar rearrangement process.

A direct proof that this rearrangement is taking place during the hydrolysis of lecithins or other glycerolphospholipids was not possible, as the necessary

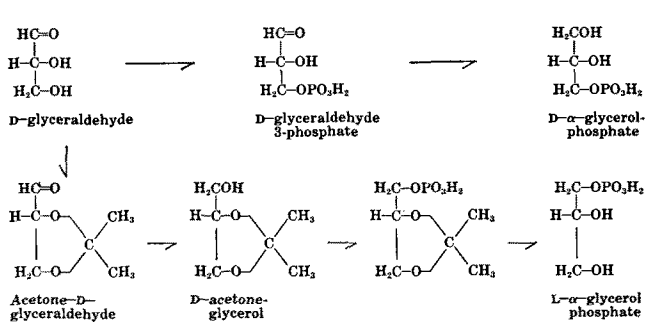


FIG. 3. Configuration of α-glycerolphosphate obtained from D-acetone glycerol. Fischer and Baer (1937).

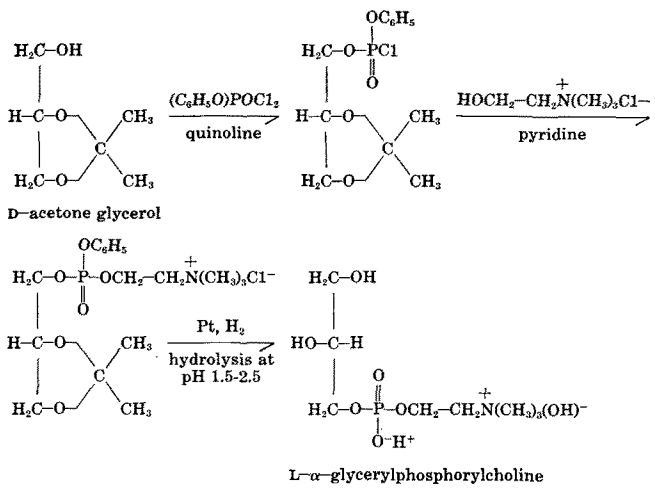


FIG. 4. Synthesis of L-α-glycerylphosphorylcholine. (Baer and Kates (1948).

substrates, i.e. pure, individual phospholipids of known structure and configuration, were not yet available. It occurred to us, however, that the same information may be obtained by studying the acid and alkaline hydrolysis of the fatty acid-free moieties of the glycerolphospholipids, i.e. α-glycerylphosphorycholine, α-glycerylphosphorylethanolamine and α-glycerylphosphorylserine. The preparation of α-glycerylphosphorylcholine from beef pancreas autolysates had been described by Schmidt, Hershman and Thannhauser (15). By following their method we obtained a satisfactory product. Needing, however, larger amounts of α-glycerylphosphorylcholine we developed a procedure for its synthesis (16). The procedure which is described by Figure 4 yields the L-isomer of α-glycerylphosphorylcholine. On subjecting the synthetic L-α-glycerylphosphorylcholine to either acid or alkaline hydrolysis (17), and analyzing the resulting mixture of glycerolphosphoric acids, we found that the acid hydrolysis yields an equilibrium mixture consisting of 88% α- and 12% of β-glycerolphosphoric acid, and the alkaline hydrolysis gives a mixture of 44% of α- and 56% of β-glycerolphosphoric acid (Table I). Similar mixtures of α- and β-glycerolphosphoric acid were obtained on acid and alkaline hydrolysis of synthetic L-α-glycerylphosphoryethanolamine (18), and synthetic L-α-lecithins (19) and L-α-cephalins (18). The ratios of α- to β-glycerolphosphoric acid in these mixtures resemble closely those reported for the hydrolysis products of natural lecithins. Furthermore, the L-α-glycerolphosphoric acid in the hydrolysis products of synthetic and natural phospholipids was racemized to a similar extent. Thus, our suspicion

TABLE I

Phosphoric Acid Migration

Glycerophosphoric acid	Acid hydrolysis	Phosphatide 100% alpha	Alkaline hydrolysis	Glycerophosphoric acid
% alpha[a]	% beta			% alpha[a] % beta
88	1 N HCl 12←110 hr 37C	Glyceryl-phosphoryl-choline	1 N NaOH 144 hr 37C	→44 56
84	1 N HCl 16←110C bath 7 hr	Lecithin	0.5N $Ba(OH)_2$ 110C bath 3 hr	→46 54
89	1 N HCl 11←110C bath 7 hr	Glyceryl-phosphoryl-ethanolamine	0.5N $Ba(OH)_2$ 110C bath 3 hr	→46 54
90	1 N HCl 10←110C bath 7 hr	Cephalin	0.5N $Ba(OH)_2$ 110C bath 3 hr	→55 45

[a] The alpha-glycerophosphoric acid is partially racemized..

that the β-glycerolphosphoric acid in the hydrolysis products of natural lecithins and cephalins is the product of phosphoric acid-migration was fully confirmed. The concept of the occurrence of β-lecithins and β-cephalins in nature, may I remind you, was based solely on the presence of β-glycerolphosphoric acid in the hydrolysis products of natural phospholipids. It is thus obviously no longer tenable.

The synthesis of L-α-glycerylphosphorylcholine and L-α-glycerylphosphorylethanolamine (20), which made it possible to compare their optical rotations with those of the corresponding compounds obtained from natural sources, had provided us with yet another simple method for the elucidation of the structure and configuration of natural glycerolphospholipids. A comparison revealed that glycerylphosphorylcholine and glycerylphosphorylethanolamine obtained from natural sources had the same specific rotation as synthetic L-α-glycerylphosphorylcholine and L-α glycerylphosphorylethanolamine, thus confirming our provisional assignments of the α-structure and L configuration to natural lecithins and cephalins.

Having established with virtual certainty the structure and configuration of two of the major classes of natural glycerolphospholipids, we were naturally very much interested in their synthesis, especially, in view of the fact that practically nothing was known regarding the chemical and physical properties of pure, individual glycerolphospholipids. Most of this information had been obtained by studying mixtures of phospholipids that at best could be called pure only in the sense that all of the components were of the same group. The synthesis of pure, individual phospholipids of assured structure and configuration thus appeared to us of fundamental importance to chemical and biochemical research in the phospholipid field.

Various attempts to obtain individual lecithins and cephalins by synthesis had been made from time to time. Most of these attempts were unsuccessful. We know now that neither the materials nor the methods were likely to give the desired compounds (21). The first reliable syntheses of cephalins were reported by Rose (22) and Hunter et al. (23). None of the earlier methods, however, permitted the synthesis of the enantiomeric forms of glycerolphospholipids. Such methods had yet to be developed. It was highly desirable that these methods should reveal the stereochemical relationship of the synthetic phospholipids to D- and L-glyceraldehyde, the stereochemical compounds of reference, as this would make it possible to assign a definite configuration to the synthetic and subsequently to the natural glycerolphospholipids.

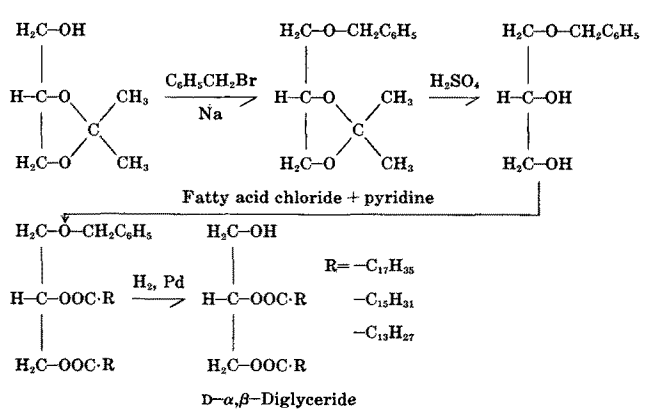


FIG. 5. Synthesis of D-α,β-diglycerides. Fischer and Sowden (1941).

The remainder of my talk thus will deal both with a discussion of our synthetic methods by means of which we have prepared representatives of most major classes of natural glycerolphospholipids, and a confirmation of the α-structure and L-configuration of natural phospholipids by direct comparison of the corresponding natural and synthetic compounds.

To begin with, I would like to show you our procedures for the synthesis of L-α-lecithins and L-α-cephalins. Both procedures require as starting materials D-α, β-diglycerides. Their preparation from D-acetone glycerol by the method of Sowden and Fischer (24) is outlined by Figure 5. D-acetone glycerol is converted to the benzyl ether by means of sodium and benzyl bromide. The protective acetone group then is removed by acid hydrolysis, and the fatty acids are introduced by acylation with fatty acid chlorides and pyridine. Removal of the benzyl group by catalytic hydrogenolysis yields the desired D-α,β-diglycerides. These are phosphorylated as shown by Figure 6 with phenylphosphoryl dichloride and pyridine. Without separating the mixture of reaction products, the phenyl ester of the phosphatidic acid chloride is esterified with choline chloride, and the resulting phenyl ester of the lecithin is freed of the protective phenyl group by catalytic hydrogenolysis. In this manner, we have prepared a series of homologous L-α-lecithins with fatty acid substituents ranging from 6 to 18 carbon atoms (25–28). The synthetic L-α-(dipalmitoyl) lecithin and a dextrorotatory dipalmitoyl-lecithin that had been isolated by Anderson and Lesuk (29) from *Cysticercus fasciolaris*, and by Thannhauser and colleagues (30,31) from lung, spleen and brain of ox were found by us to be identical substances. The natural phospholipid thus possesses an α-structure and L-configuration. It was the first natural phospholipid to be obtained by synthesis. The same structure and configuration was assigned by us to lecithin of egg yolk, since on catalytic reduction it gives a distearoyl lecithin which is identical in

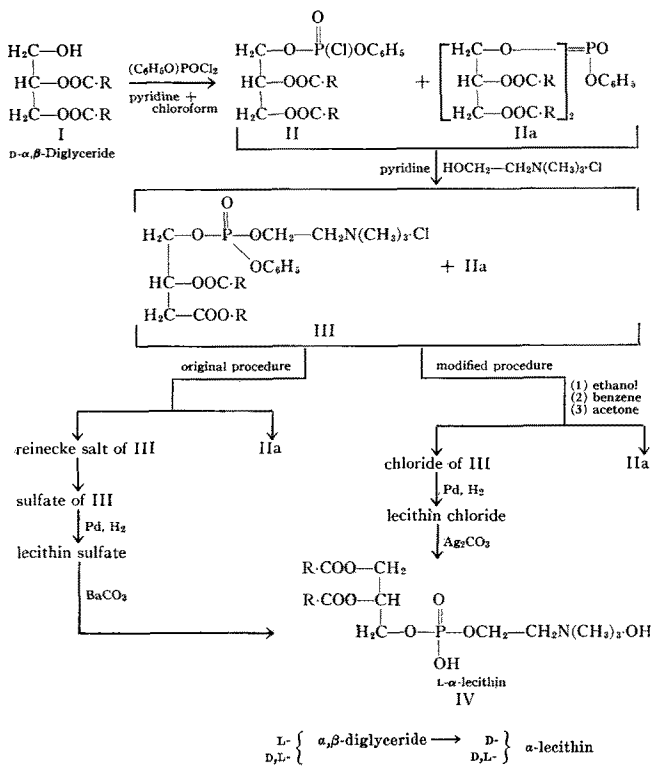


FIG. 6. Synthesis of L-α-lecithins. Baer and Kates (1950).

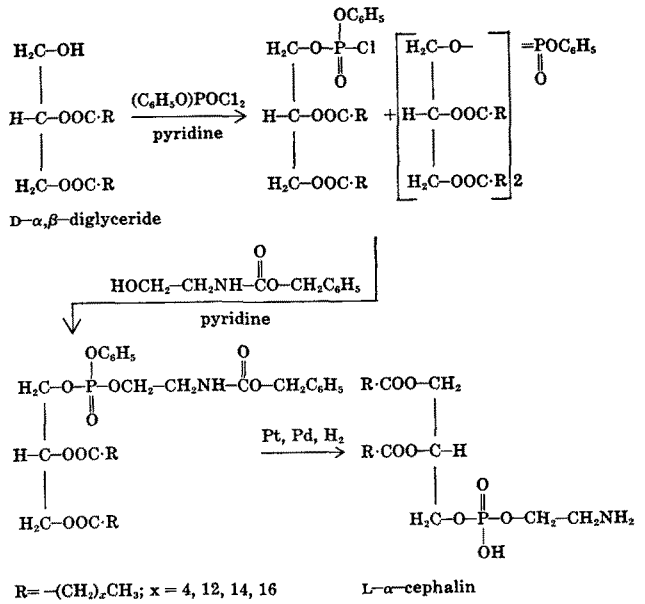


FIG. 7. Synthesis of L-α-cephalins. Baer, Maurukas and Russell (1951–1952).

all respects with our synthetic L-α-(distearoyl) lecithin. Thus, for the first time in the history of the phospholipids a definite structure and configuration has been assigned to two of their members.

Our procedure for the synthesis of L-α-cephalins (32,33) resembles that for the lecithins, except that ethanolamine replaces choline (Fig. 7). To prevent any interference of the amino group with phosphorylation, it was protected with the carbobenzoxy group. The simultaneous removal of the phenyl and carbobenzoxy groups by catalytic hydrogenolysis gave cephalin. Again, a series of L-α-cephalins was prepared with stearic-, palmitic-, myristic and caproic acid as substituents. One of this series, namely L-α-(distearoyl) cephalin, and a cephalin isolated by Levene and West (34) from a hydrogenated mixture of egg yolk-phospholipids were found to be identical compounds. Thus cephalins of egg yolk, as well as the lecithins possess the α-structure and L-configuration.

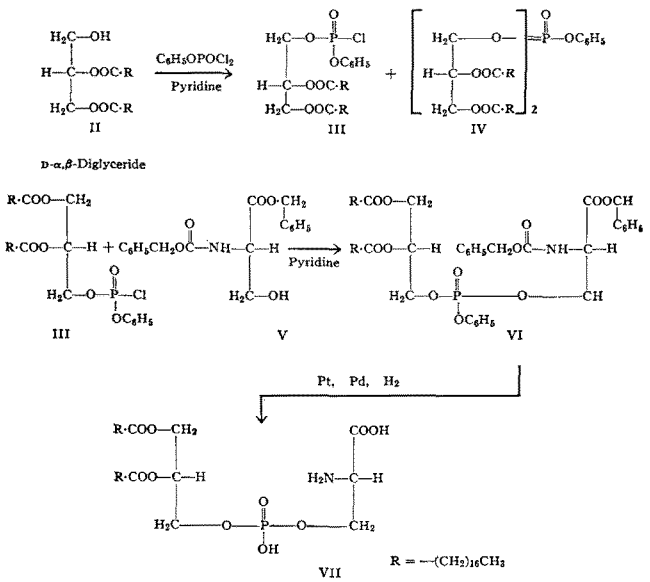


FIG. 8. Synthesis of L-α-phosphatidyl-L-serine. Baer and Maurukas (1955).

In a similar manner, we undertook the elucidation of the structure and configuration of phosphatidyl serines. The synthesis of this group of naturally occurring phospholipids, which were discovered by Folch (35), was somewhat more difficult than those of lecithin and cephalin because of the presence of the carboxyl group of serine. It, like the amino group, had to be inactivated by a protective group to prevent its interference with the phosphorylation procedure. Further complications were introduced by the fact that phosphatidyl serines possess two asymmetric centers and thus, theoretically at least, can occur in nature in either one of four stereoisomeric forms. Our procedure for the synthesis of phosphatidyl serines (36,37), in particular of L-α-phosphatidyl-L-serines, the most likely of the four isomers to occur in nature, is outlined by Figure 8. Again, we phosphorylated D-α,β-distearin with phenylphosphoryldichloride and pyridine, and without separating the reaction mixture we esterified the phenyl ester of the phosphatidyl chloride with the benzyl ester of N-carbobenzoxy L-serine. The simultaneous removal of the three protective groups by catalytic hydrogenolysis gave distearoyl L-α-glycerylphosphoryl-L-serine. This compound and the reduction product of phosphatidyl serine of ox brain, which we had isolated by the procedure of Folch, proved to be identical substances. The phosphatidyl serines thus are the third major class of natural phospholipids to possess the α-structure and L-configuration. Since the plasmalogens also possess this structure and configuration, it appears to be fairly certain now that the α-structure and L-configuration is common to all naturally occurring glycerolphospholipids.

By substituting seryglycylglycine (38,39) for serine

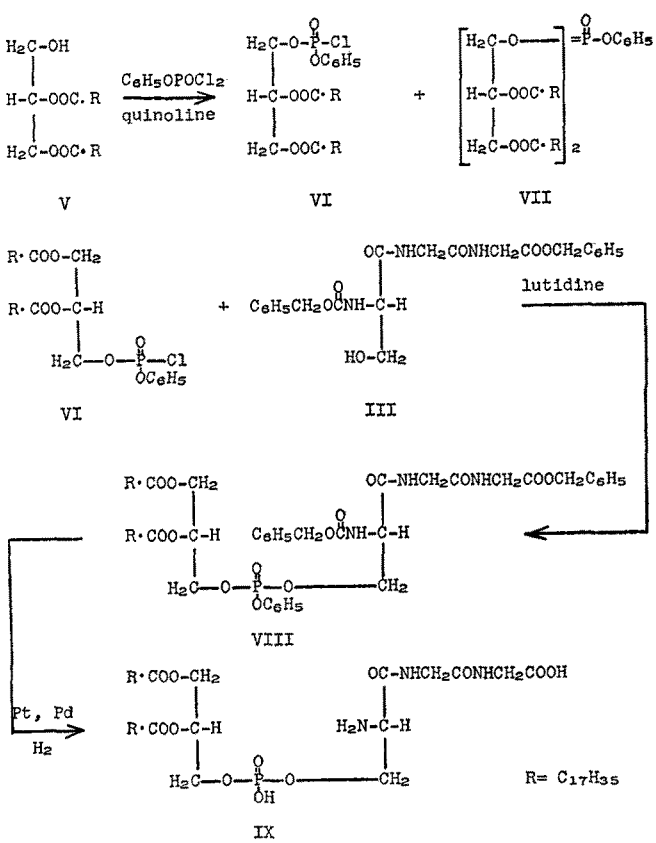


FIG. 9. Synthesis of phosphatidyl peptides. Baer, Maurukas, and Clarke (1957).

in the procedure for the synthesis of phosphatidyl serine, a phosphatidyl tripeptide (39) was obtained. Its synthesis is illustrated by Figure 9. It is the first representative of a new and still somewhat controversial type of phospholipids that appears to occur in nature. I shall not go into the details of its synthesis beyond stating that our original procedure required the preparation of 22 intermediates. Many of these were required for the preparation of the tripeptide.

By methods similar to those just described, we have prepared in recent years a phosphatidyl threonine (40) and a phosphatidyl hydroxyproline (41), whose structures are shown by formulae I and II of Table II. These two phospholipids were synthesized believing that they may prove to be biological compounds. Our expectations were at least partially realized by the recent discovery of a phosphatidyl threonine in both egg yolk (42) and the muscle of tuna fish (43). Furthermore, we synthesized cephalins whose amino groups were substituted by either one or two methyl groups. The general structure of these phospholipids is shown by formulae III and IV of Table II. The N-monomethyl- (44) and N,N-dimethylcephalins (45) are of considerable interest to biochemists as they appear to be intermediates in the biosynthesis of lecithins from cephalins by the progressive methylation of the amino group.

The general structures of yet two other classes of phospholipids, i.e. the phosphatidyl-2-amino-1-propanols (46) and phosphatidyl-2-methylpropanols (47), members of which were synthesized by us for the first time, are shown by formulae V and VI of Table II. The phosphatidyl-2-amino-2-methylpropanol is the first representative of a newly discovered class of phospholipids that are formed in nature under special circumstances. Evidence for their in vivo formation by incorporation of 2-amino-2-methylpropanol into rat liver phospholipids has recently been reported by Longmore and Mulford (48).

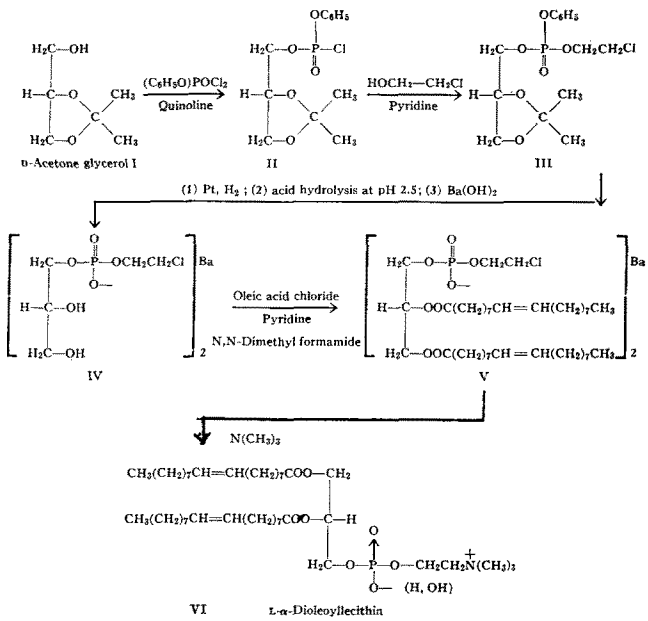


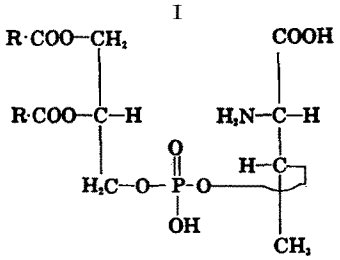
FIG. 10. Synthesis of L-α-dioleoyllecithin. Baer, Buchnea and Newcombe (1956).

Up to now, I have discussed only the synthesis of phospholipids with two identical saturated fatty acid substituents. However, phospholipids containing two identical unsaturated fatty acids occur in nature also, as Hanahan (49) has shown. These unsaturated phospholipids can not be synthesized by the procedures I have just discussed, as the removal of the protective groups by catalytic hydrogenolysis in the final stages of the synthesis would bring about the reduction of the unsaturated fatty acids. To obtain unsaturated phospholipids new procedures had to be devised.

Figure 10 illustrates our first procedure for the synthesis of L-α-(dioleoyl) lecithin (50). To obtain

TABLE II

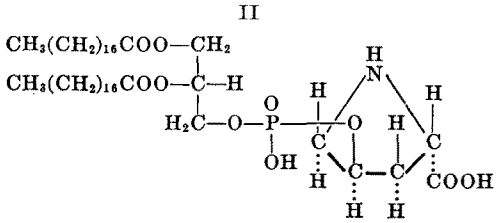
I



Phosphatidylthreonines

E. Baer and F. Eckstein (1962)

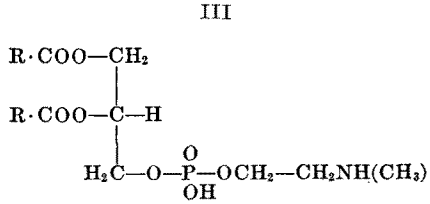
II



Phosphatidylhydroxyproline

E. Baer and A. Zschocke (1961)

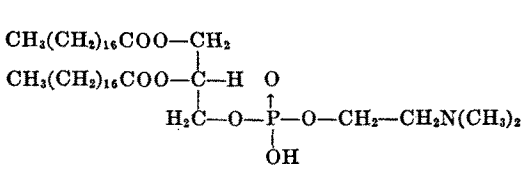
III



N-Methylcephalins

E. Baer and S. K. Pavanaram (1961)

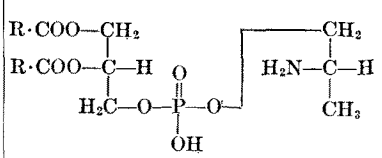
IV



N,N-Dimethylcephalins

E. Baer and S. K. Pavanaram (1961)

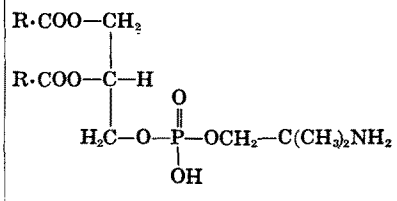
V



L-α-Phosphatidyl-L-2-amino-1-propanol

E. Baer and J. Blackwell (1963)

VI



Phosphatidyl-2-amino-2-methylpropanols

E. Baer and G. V. Rao (1963)

the unsaturated phospholipid we phosphorylated D-acetone glycerol with phenylphosphoryl dichloride and quinoline, and esterified the resulting acetone L-α-glyceryl(phenyl)phosphoryl chloride with ethylene chlorohydrin in the presence of pyridine. The reaction product then was freed of its productive phenyl group by catalytic hydrogenolysis, and of its acetone group by mild acid hydrolysis, and the L-α-glyceryl-phosphoryl ethylene chlorohydrin thus obtained was converted to its barium salt, and acylated with oleoyl chloride. Treatment of the reaction product with trimethylamine gave L-α-(dioleoyl) lecithin.

Somewhat later we succeeded in devising a procedure for the synthesis of unsaturated lecithins (51)

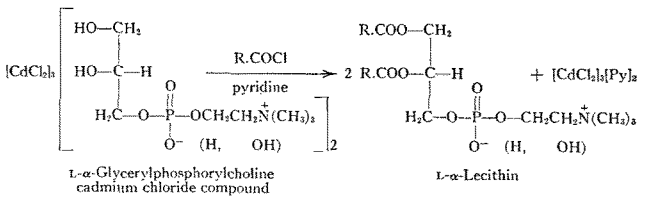


FIG. 11. Baer and Buchnea (1959).

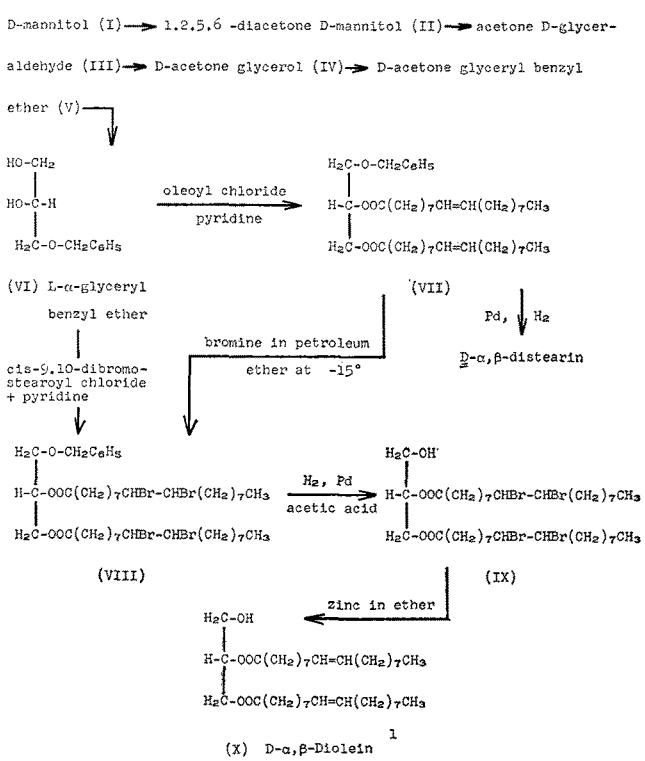


FIG. 12. Synthesis of D-α,β-diolein. Baer and Buchnea (1958).

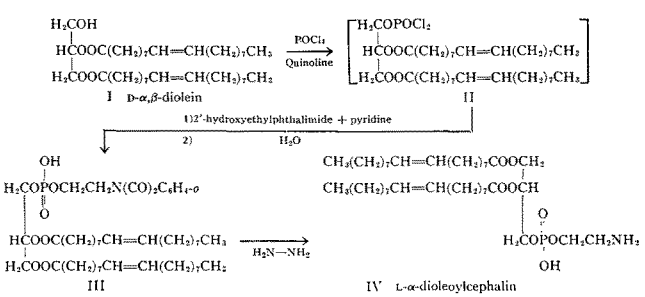


FIG. 13. Synthesis of L-α-(dioleoyl)cephalin. Baer and Buchnea (1959).

which was not only simpler but permits also the synthesis of saturated lecithins. Figure 11 shows the principle of the method. The lecithins are obtained by acylating L-α-glycerylphosphorylcholine, in the form of its cadmium chloride complex, with the chloride of a saturated or unsaturated fatty acid in the presence of pyridine. The acylation proceeds rapidly at low temperature and gives in fairly good yields the saturated or unsaturated L-α-lecithin.

The synthesis of an unsaturated α-cephalin, viz. L-α-(dioleoyl)cephalin (52), was made possible by the fact that we had succeeded in preparing D-α,β-diolein. The procedure for its preparation, which was developed by Buchnea (53), is outlined by Figure 12. The diolein was obtained by acylating L-α-glyceryl benzyl ether with 9,10-dibromostearic acid chloride and pyridine, removing the benzyl group of the diglyceride by catalytic hydrogenolysis, and forming the double bond of the oleic acid by treating the tetrabromo-distearin with activated zinc. Phosphorylation of D-α,β-diolein (Figure 13) with phosphorus oxychloride and quinoline, esterification of the phosphatidyl dichloride with N-phthaloyl ethanolamine, removal of the phthaloyl group of N-phthaloylcephalin with hydrazine, and purification of the reaction product by column chromatography gave analytically pure L-α-(dioleoyl)cephalin.

More recently, we succeeded in developing a procedure for the synthesis of saturated as well as unsaturated α-cephalins (54,55) which is considerably shorter than any of the known procedures for their synthesis because it does not require diglycerides or their derivatives as starting materials. The cephalins (Fig. 14) were obtained by phosphorylating D-, L-, or DL-acetone glycerol with phosphorus oxychloride and quinoline, esterifying the resulting acetone α-glycerylphosphoric acid dichlorides with 2-hydroxyethylphthalimide, removing the acetone group by mild acid hydrolysis, acylating the barium salt of D-, L-, or DL-α-glycerylphosphoryl-2-hydroxyethylphthalimide with the chloride of the saturated or unsaturated fatty acid, and removing the phthaloyl group by hydrazinolysis. In this manner we prepared L-α-cephalins containing stearic-, palmitic-, oleic- and linoleic acid.

Phospholipids containing two identical fatty acid substituents, whether saturated or unsaturated, have been isolated from natural sources in a few instances only. In general, natural phospholipids appear to possess two dissimilar fatty acid substituents, one of which in most cases is unsaturated. The chemical synthesis of phospholipids with two dissimilar fatty acid substituents, one of them preferably unsaturated, thus was one of the outstanding major problems remaining in this field.

The synthesis of mixed-acid phospholipids by our methods requires as starting materials mixed-acid

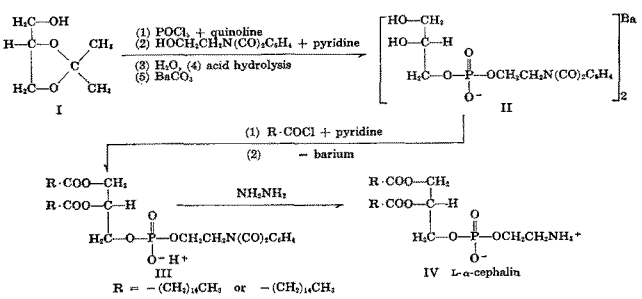


FIG. 14. Synthesis of α-cephalins by a new procedure. Baer, Suzuki and Blackwell (1963); Baer and Blackwell (1964).

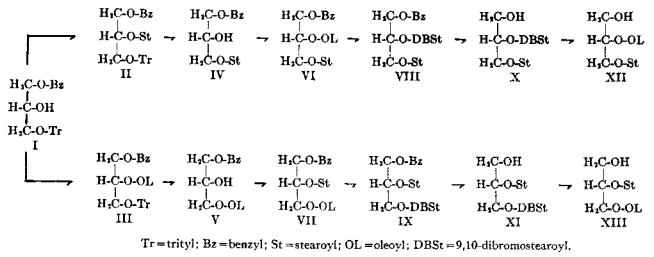


FIG. 15. Synthesis of enantiomeric mixed-acid α,β-diglycerides. Baer and Buchnea (1960).

diglycerides. Since these were not yet available, a procedure for their synthesis had to be devised. This was accomplished by my colleague Buchnea (56). Time, however, does not permit me to elaborate on this elegant procedure which is outlined by Figure 15. With the required starting materials available, we were now able to undertake the synthesis of mixed-acid glycerolphospholipids (57). Figure 16 illustrates our procedure for the synthesis of both α'-stearoyl,β-oleoyl- and α'-oleoyl,β-stearoyl L-α-cephalins. They were obtained by the same procedure which gave us L-α-(dioleoyl)cephalin, except that now D-α'-stearoyl,β-oleoyl-glycerol and D-α'-oleoyl,β-stearoyl glycerol were used as starting materials. Phosphorylation of the two diglycerides with phosphorus oxychloride and quinoline gave the corresponding phosphatidic acid chlorides, which on esterification with phthaloylethanolamine in the presence of pyridine formed the N-phthaloylcephalins. These, on treatment with hydrazine gave α'-stearoyl,β-oleoyl L-α-cephalin and α'-oleoyl,β-stearoyl L-α-cephalin, respectively. The two cephalins are remarkable for the fact that their glyceride moieties are positional isomers. Since both cephalins are devoid of contamination by other positional or spatial isomers, and furthermore contain two of the most widely occurring fatty acids in nature, they are ideal substrates for enzymatic studies. There is a strong possibility that either one of the two cephalins, perhaps both, may occur in nature.

In addition to the phospholipids whose syntheses I have described, a considerable number of other phosphoric acid esters were prepared in my laboratory (Table III). These include the phosphatidic acids and bis-phosphatidic acids with two identical saturated or unsaturated fatty acids (58–60) as well as two dissimilar fatty acid substituents (unpublished work), saturated and unsaturated phosphatidyl glycerols (61), a polyglycerylphosphoric acid (62), and the fatty acid-free moieties of phosphatidyl serine (63) and phosphatidyl-2-amino-2-methylpropanol (64). These compounds are all of considerable interest as possible intermediates in phospholipid metabolism. We have also prepared two amino acid esters of a phosphatidyl glycerol (unpublished work) which represents a group of phospholipids that only recently has been found to occur in nature (65). Their synthesis is shown by Figure 17. The procedure has an interesting feature to which I would like to draw your attention. It is the novel manner with which it introduces the amino group into a phospholipid. The amino group is formed in the final step of the synthesis by the catalytic reduction of the azido group, and proceeds concurrently with the removal of the protective phenyl and carbobenzoxy groups by hydrogenolysis. The use of the "self-protecting" azido group eliminates the necessity of preparing

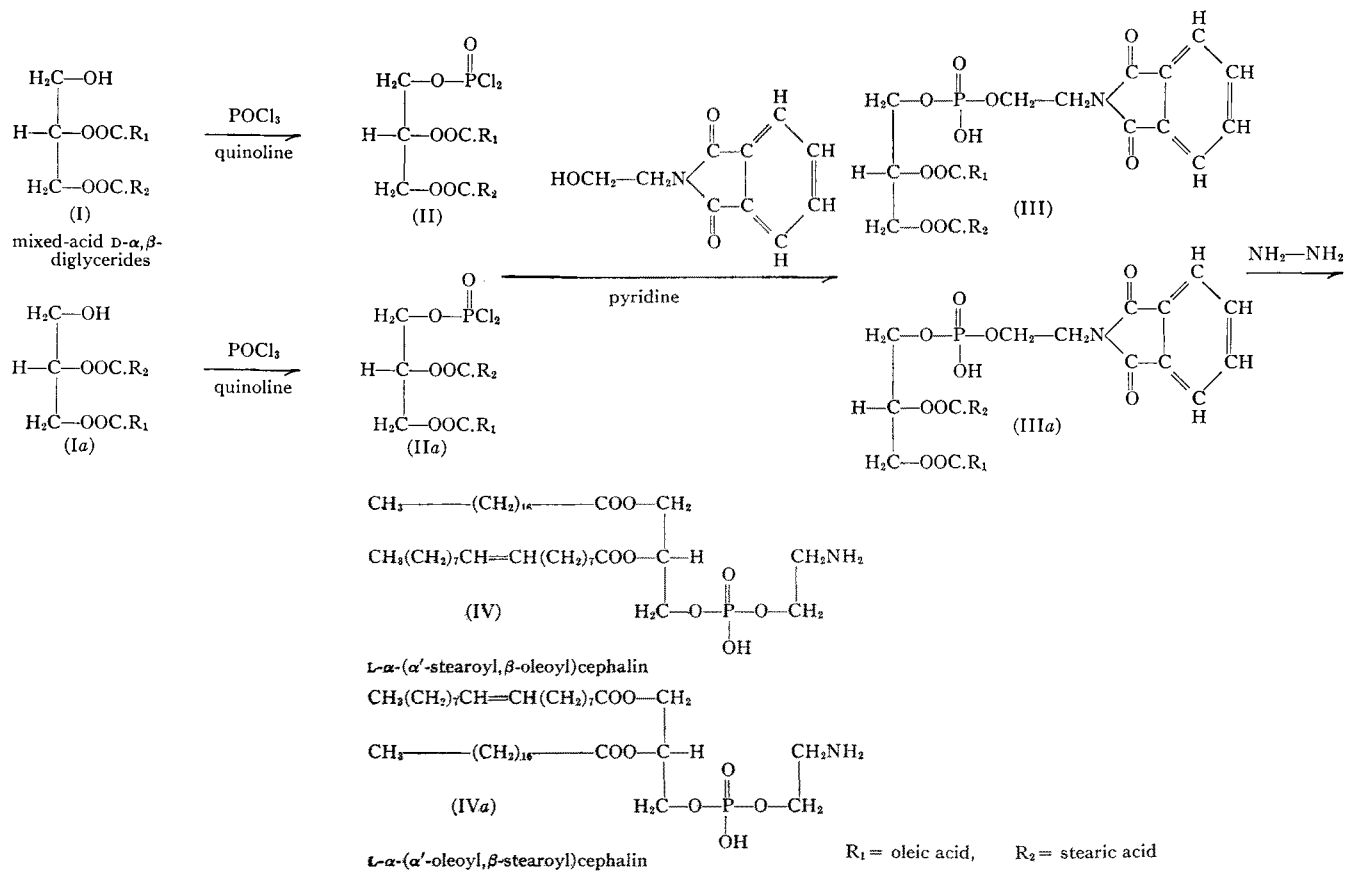
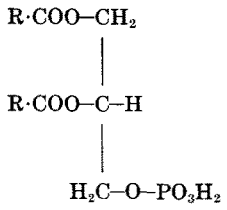
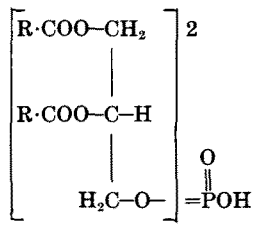
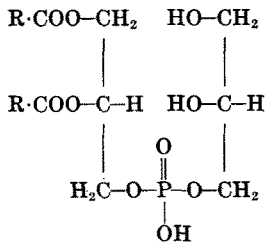
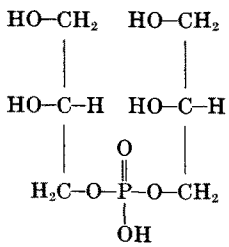
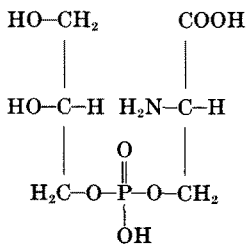
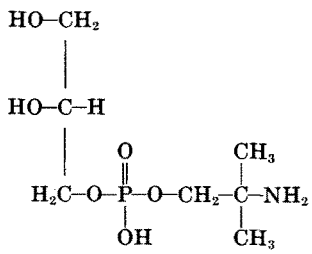


FIG. 16. Synthesis of mixed-acid cephalins. Baer and Buchnea (1961).

TABLE III

 2 ident. sat. F. acids (1951) 2 ident. unsat. F. acids (1958) 2 dissimilar F. acids (1965) L-α-Phosphatidic Acids	 2 ident. sat. F. acids (1958) 2 ident. unsat. F. acids (1958) 2 dissimilar F. acids (1965) L-α-Bisphosphatidic Acids	 Sat. and unsat. F. acids (1958) L-α-Phosphatidyl-L-α-glycerol
 Bis-(L-α-glyceryl)-phosphoric acid (1958)	 L-α-Glycerylphosphoryl-L-serine (1959)	 L-α-Glycerylphosphoryl-2-amino-2-methylpropanol (1965)

several intermediates that otherwise would be required to obtain the amino acid esters of β-benzyl glycerol with a suitably blocked amino group.

The discovery of new types of phospholipids in biological material is by no means at an end. This has been forcefully demonstrated in recent years. Thus, for instance the isolation of dialkyl glycerol ethers from the hydrolysis products of beef heart phospholipids by Marinetti et al. (66), and from the phospholipids of the *Halobacterium cutirubum* by Sehgal et al. (67) suggests strongly the natural occurrence of phospholipids containing two ether- instead of two ester groups in their glyceride moiety. At present we are undertaking the synthesis of diether-analogues of the major groups of glycerolphospholipids. Formula I (Table IV) shows the structure of a diether analogue of a lecithin (68) which was prepared by my colleague, Dr. Stanacev. He obtained the material by a procedure which was developed in our laboratory for the synthesis of ester-lecithins (19), replacing, however, the ester-glyceride as starting material by an ether-glyceride.

Entirely unexpected and most surprising has been the recent isolation of 2-aminoethylphosphonic acid from biological materials. This unusual phosphorus-compound was obtained from the hydrolysis products of proteolipid-like fractions of ciliate protozoa from

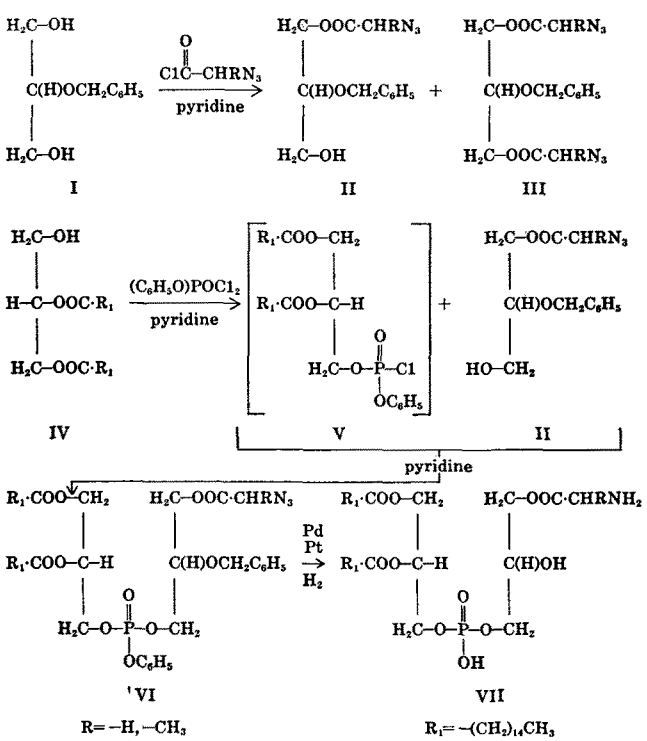
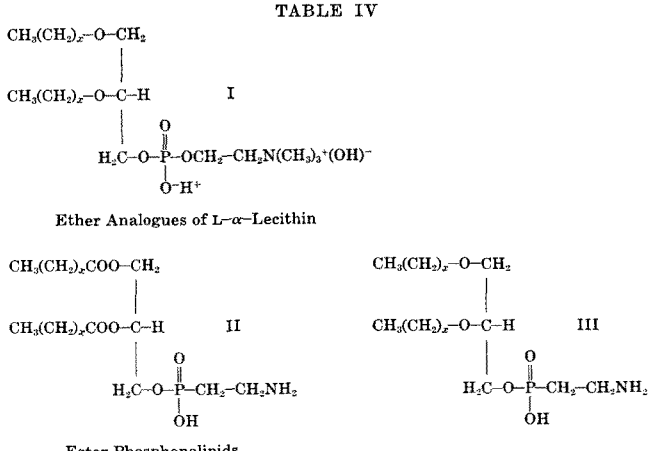


FIG. 17. Synthesis of amino acid esters of L-α-phosphatidyl-α'-glycerol. Baer and Rao (1964, unpublished work).

TABLE IV



Baer and Stanacev (1964/65)

sheep rumen by Horiguchi and Kandatsu (69), and the hydrolysis products of ethanolic extracts of the sea anemone *Anthopleura elegantissima* by Kittredge et al. (70). Its isolation from these sources is a strong indication of the natural occurrence of phosphorus-containing lipids in which phosphoric acid is replaced by phosphonic acid. We have proposed for the latter compounds the generic name "*Phosphonolipids*". The isolation of an intact phosphonolipid from the sea anemone *Anthopleura elegantissima* has been reported by Rouser et al. (71).

The synthesis of phosphonolipids which contain a carbon to phosphorus bond offers new challenges to the ingenuity of the synthetic chemist. Unfortunately, time will not allow me to discuss in detail work which we have carried out in this field. Hence, I will restrict myself to showing merely the structural formulae of some of the phosphonolipids which we have synthesized thus far. Formula II (Table IV) shows the structure of a phosphonic acid analogue of a cephalin (72). It differs from the normal cephalin in that its basic moiety is attached to the phosphorus by a carbon to phosphorus bond, which is extremely difficult to cleave by acid or alkaline hydrolysis. Formula III (Table IV) shows the structure of a phosphonic acid analogue of a diether cephalin (73). This phosphonolipid is a most interesting compound because of its peculiar stability: both its carbon to phosphorus bond and its ether bonds are highly resistant to hydrolysis by acid or alkali.

The phosphonolipids because of their chemically inert nature, and their close structural resemblance to the phospholipids should make interesting substrates for the investigation of a variety of biological reactions. For the same reason, it is not unlikely that they may possess useful therapeutic properties.

In the past hour, I have taken you over a long trail of research, remarkable perhaps for its straightness. It has led from a study of the chemical properties of the trioses and their derivatives to the synthesis of glycerolphospholipids and their biological intermediates. You have been shown how our early work in the carbohydrate field not only has provided the starting materials for the synthesis of asymmetrically substituted glycerol derivatives in both of their enantiomeric forms, but also has laid the theoretical foundations for the stereochemistry of the glycerolphospholipids. It has linked the phospholipids to the sterochemical reference compounds in carbohydrate chemistry, i.e. D- and L-glyceraldehyde, and thus has made it possible to assign specific configurations to the phospholipids. Furthermore, the synthesis of members of several major classes of naturally occurring phospholipids by a variety of synthetic procedures has been described. In all cases, these syntheses were the first for these types of compounds. Not only were they instrumental in the elucidation of the structure and configuration of naturally occurring glycerolphospholipids, but they also provided for the first time synthetic standards by which the purity of natural phospholipids could be judged. The synthetic compounds made it possible to obtain the first reliable data on the physical and chemical properties of individual phospholipids, and moreover were the first pure, individual phospholipids to be available for biological research.

In closing, I would like to emphasize that even today, in spite of the tremendous progress that has been made in recent years in the separation and purification of naturally occurring phospholipids, *synthesis,* in most cases still offers the only means of obtaining useful amounts of pure, individual phospholipids of known structure and configuration.

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