# THE SYNTHESIS OF PHOSPHOLIPIDS.

by

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Thesis submitted to the University of Bristol

for the Degree of Doctor of Philosophy.

September 1958.

MEMORANDUM.

This dissertation is based on work carried out independently by the candidate, P. J. C. Counsell, under the supervision of Dr. T. Malkin, D.Sc., Ph.D., Reader in Organic Chemistry at the University of Bristol.

A short synopsis of the work is included in the dissertation, which is divided into Theoretical and Experimental sections. The former contains a general review of current knowledge in the phospholipid field, with regard to structure, occurrence, isolation from natural sources and synthesis.

The Experimental section comprises two separate Parts, (1 and 11). Part 1 deals with the synthesis of optically active cephalins and phosphatidyl serine. This work was carried out using methods develpped in these laboratories for the corresponding optically inactive compounds, with the purpose of clarifying certain anomalies in the literature regarding the melting points of the above mentioned classes of glycerophosphatides. The properties of the synthetic compounds obtained by the author have been described.

Part 11 of the Experimental section concerns the attempted synthesis of lecithins and cephalins containing unsaturated fatty acids. Two methods for the preparation of unsaturated cephalins have been developed, but one of them has been satisfactorily concluded for a saturated compound only, owing to experimental difficulties and lack of time. Further studies

would doubtless permit the synthesis of unsaturated compounds.

The author had no direct success with the lecithins, although approaches meriting further investigation have been devised. It is felt that this field offers much scope to future workers.

P. J. B. Counsell.

18th September 1958.

## SYNOPSIS

This dissertation is divided into Theoretical and Experimental sections. The former contains a general review of current knowledge in the phospholipid field, with regard to structure, occurrence, isolation from natural sources and synthesis.

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# CONTENTS.

Contents (cont.). Page

Contents (cont.).

Contents (cont.).

Contents (cont.). Page

Contents (cont.). Page



Plates.

# THEORETICAL SECTION

## The Phospholipids

### Introduction

The phospholipids are an important group of compounds, widely distributed in the plant and animal kingdoms. They are thought to be of physiological importance in the transport of fat in the living organism, and to play a part in the transfer of messages from nerve cells to the brain.

The phospholipids find application in industry as emulsifying and wetting agents, and one group in particular, the lecithins, is used in the manufacture of paints, chocolates and pastry foods. Phospholipids may be incorporated into petroleum products, rubber, leather, protective coatings and printing inks. They are also used in the production of textiles and plastics, and also in pharmaceutical products.

## Chemical Constitution of the Phospholipids

The diversity of the chemical composition of the phospholipids is very wide, and it is convenient to consider them in two groups, namely the Glycerophosphatides and the Sphingolipids.

## Glycerophosphatides

These are by far the largest class of phospholipids, and they are found in the essential organs of animals and in the seeds and storage organs of plants. They are based on a pentavalent phosphorus atom esterified with a glycerol moiety, and usually further esterified with an amino alcohol or amino hydroxy acid.

The two remaining hydroxyl groups in the glycerol moiety are generally either acylated with long chain fatty acids, as in the Ester Phosphatides, or linked by an acetal grouping to a long chain aldehyde, as in the Acetal Phosphatides. These further classifications will be considered separately.

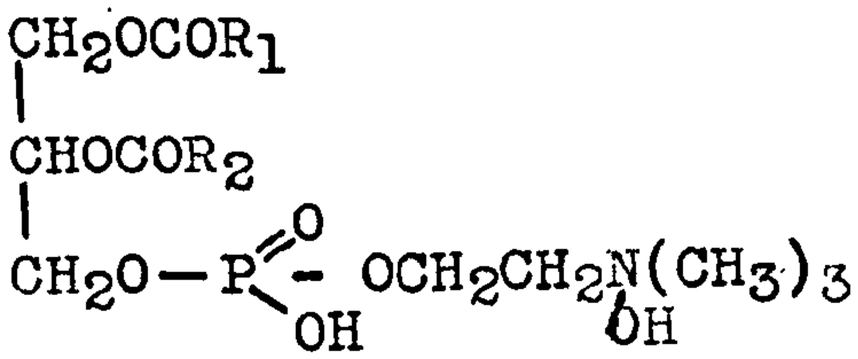
### a) Ester Phosphatides

In the following description, the trivial or common name of the group of compounds is given first, followed by

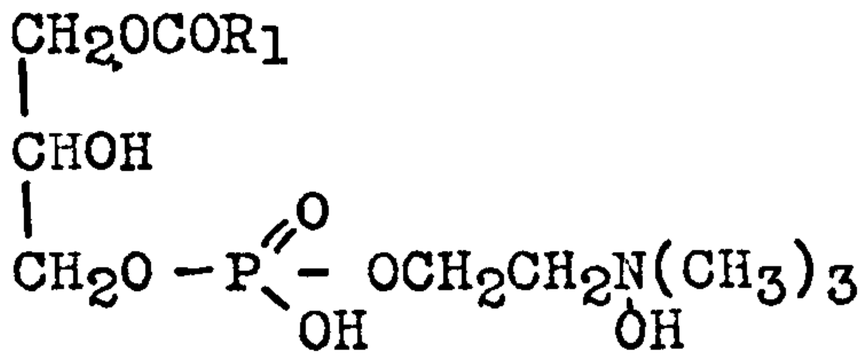
the rational nomenclature as proposed by Folch (J.B.C. 174 439 (1948) ).

Lecithins

The Lecithins, or Phosphatidyl cholines, have the following general structure:-



∝ DIACYL LECITHIN
(1,2 DIACYL LECITHIN)

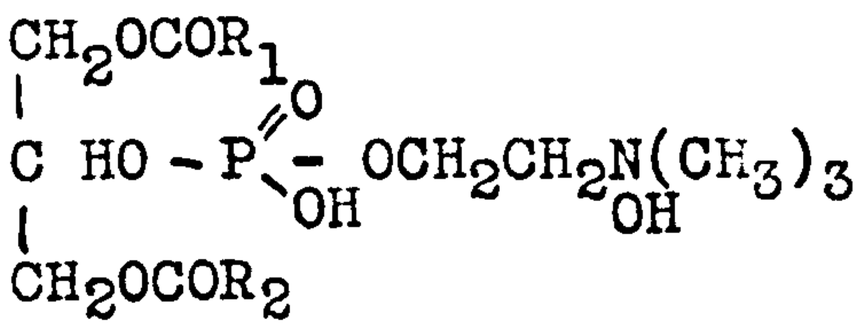


∝ "LYSO" LECITHIN

The nitrogen/phosphorus ratio is always 1/1.

The glycerol moiety is acylated at one or two of the hydroxyl groups, in the "lyso" and diacyl compounds respectively.

The phosphorus atom may be linked to the central or β hydroxyl group of the glycerol molecule, in which case the compound is a β lecithin (Sometimes called a 1,3 diacyl lecithin) e.g.

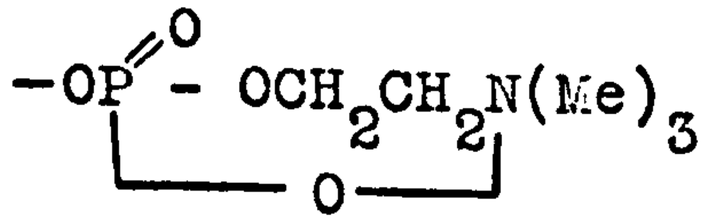


The long chain fatty acids $R_1COOH$ and $R_2COOH$ may or may not be identical, and in the naturally occurring compounds saturated (e.g. stearic acid) or unsaturated (e.g. oleic acid).

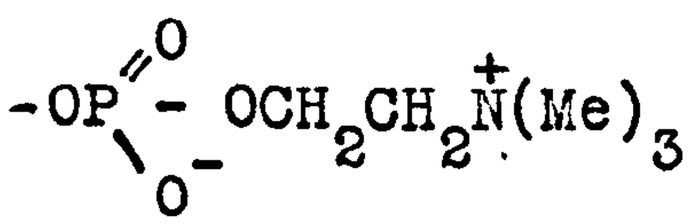
In general the fatty acids in the naturally occurring phosphatides tend to be stored in the maximum degree of unsaturation, as opposed to the glycerides where the reverse is the case. (Klenk Z. Phys. Chem. 192, 217 (1930) ); (Klenk and von Schoenbeck ibid 194 191 (1931) ) ibid 209 112 (1932).

Klenk's work showed that a complete series of even membered unsaturated acids from $C_{16}$ to $C_{24}$ existed in brain phosphatide, the $C_{18}$ and $C_{24}$ acids predominating, whereas palmitic acid ($C_{16}$) and stearic acid ($C_{18}$) were the only saturated acids present. This was also true of plant sources.(Klenk ibid 232 47 (1935) ).

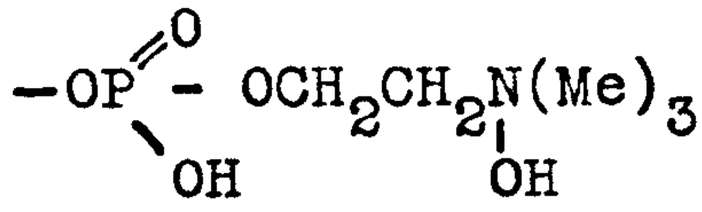
There has been some doubt as to the structure of the phosphorus - choline linkage. Grün and Limpacher (Chem. Umschau 30 246 (1923) ; Ber 59 1345 (1926) ) proposed an inner anhydride or "endo" salt formulation, viz:-



whereas Jukes (J.B.C. 107 783 (1934) ) postulated the structure as a zwitterion :-

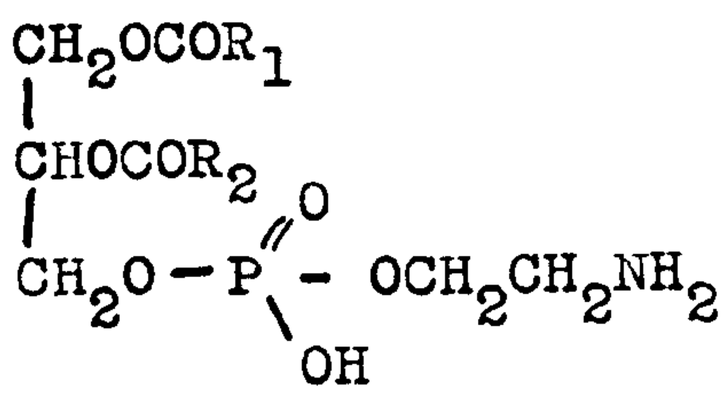


The recent work of Bevan and Malkin (in preparation) on the synthesis of lecithins suggests that the lecithins should be formulated as follows, in order to correspond with analytical results :-



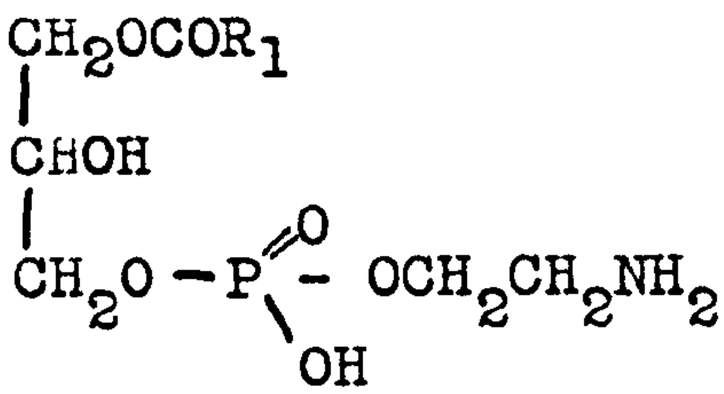
Cephalins

The cephalins (kephalins) or phosphatidyl ethanolamines are formulated as follows:-



∝ DIACYL CEPHALIN

(1,2 DIACYL CEPHALIN)



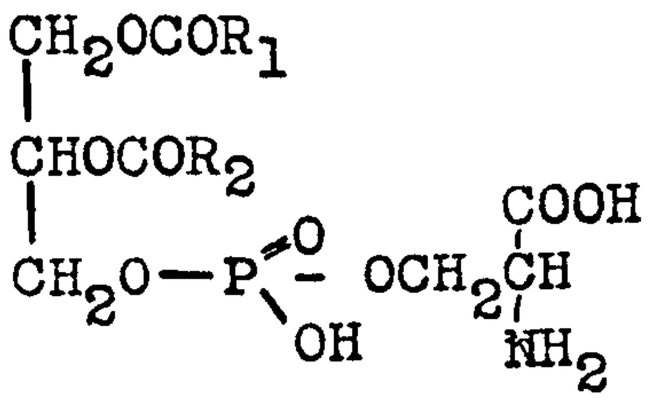
∝ "LYSO" CEPHALIN

Again the nitrogen/phosphorus ratio is 1/1.

Cephalins with a similar structure to the $\beta$ lecithins also exist. The presence of the amino group was proved by the preparation of phenyl and napthylisocyanate derivatives by Levene and West (J.B.C. 25 517 (1916b) ).
The fatty acids of the naturally occurring cephalins are in general similar to those of the lecithins.

Phosphatidyl Serine

In the phosphatidyl serines the phosphorus atom is esterified with the primary hydroxyl group of the amino acid, serine:-



∝ PHOSPHATIDYL SERINE (1,2 DIACYL PHOSPHATIDYL SERINE)

Nitrogen/phosphorus is 1/1.

No evidence has been put forward for the existence of β phosphatidyl serine or lyso phosphatidyl serine, but these compounds are presumably capable of being synthesised.

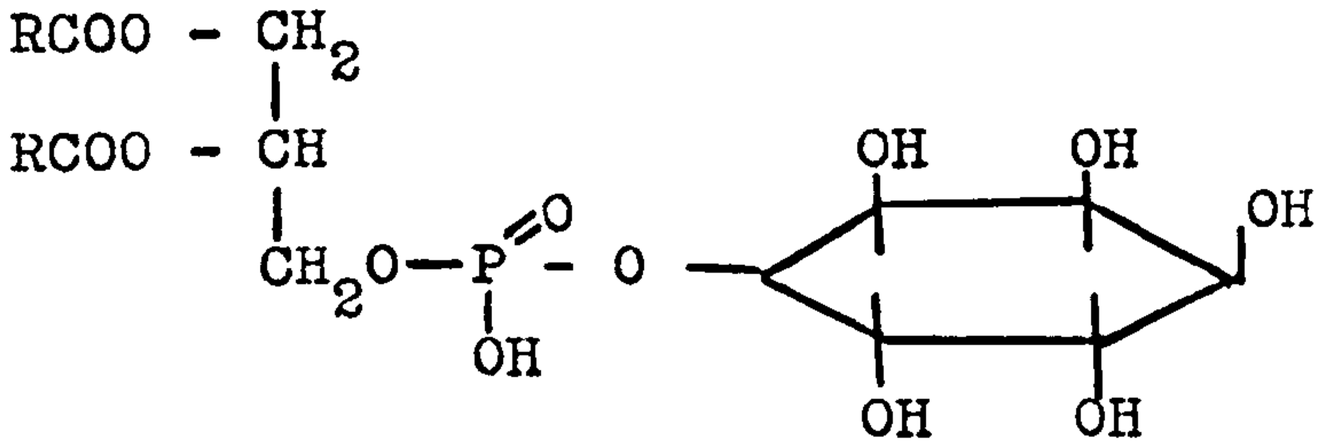
According to Folch (J.B.C. 174 439 (1948) ) the fatty acids present in natural phosphatidyl serine are predominantly stearic and oleic acids.

Phosphoglyceroinositides.

The fourth and final group of ester glycerophosphatides are the phosphoglyceroinositides. These were prepared by Folch (J.B.C. 146 35 (1942a); ibid 177 497 (1949); 177 505 (1949a) ) from brain"cephalin fraction". On addition of alcohol to a chloroform solution of the "cephalin fraction " the phosphoglyceroinositide, being the least soluble in alcohol, was precipitated first. Inorganic

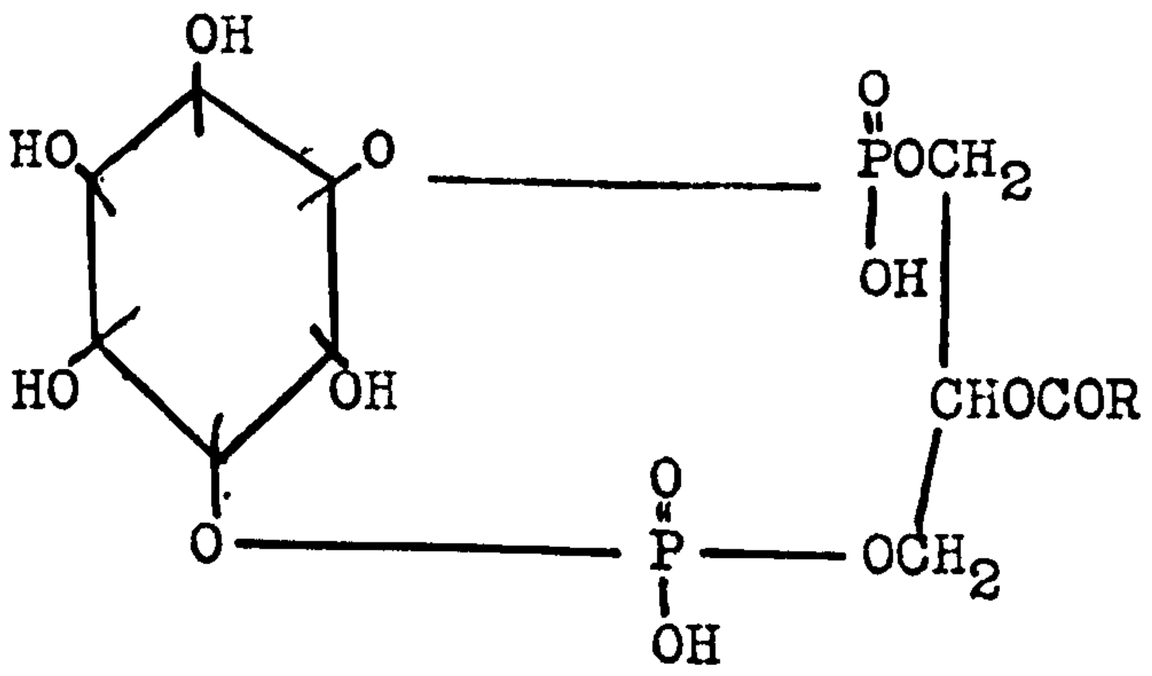
impurities were removed by dialysis.

There are two main types of phosphoglyceroinositides, and both differ from the other phosphatides in containing no nitrogen. Their formulae are as follows:-



Faure and Norelec Coulson (Compt. Rend. 236 1104 (1953) )
Malkin and Poole (J. 3470 (1953) ).

and



Hawthorne (Biochim. Biophys. Acta. 18 389 (1955) )

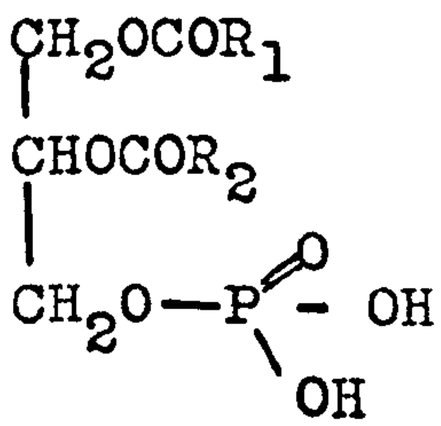
These compounds contain no nitrogen, and are acidic.

These inositol containing lipids have been isolated from soya bean (Woolley J.B.C. 147 581 (1943)) and

groundnuts (Malkin and Poole : loc cit.). They are always associated with other phosphatides, and on hydrolysis give long chain fatty acids, phosphoric acid, ethanolamine and various sugars among other products.

Minor phosphatides.

There are various minor phosphatides meriting inclusion, for example the Phosphatidic Acids, which have the following structure.

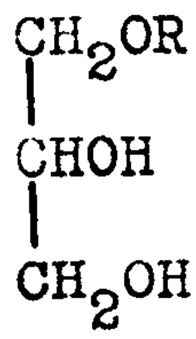


∝ DIACYL PHOSPHATIDIC ACID. (1,2 DIACYL PHOSPHATIDIC ACID ).

These substances were first discovered in " Ricinus" leaves, clover and rhubarb by Winterstein and Stegmann (Z. Physiol. Chem. 58 527 (1908/09 )), although they were not recognised as distinct compounds until 1927 when Channon and Chibnall (Biochem. J. 21 225, 233 (1927); ibid 23 176 (1929) ) isolated them from cabbage leaves. They are very similar to lecithins in their properties.

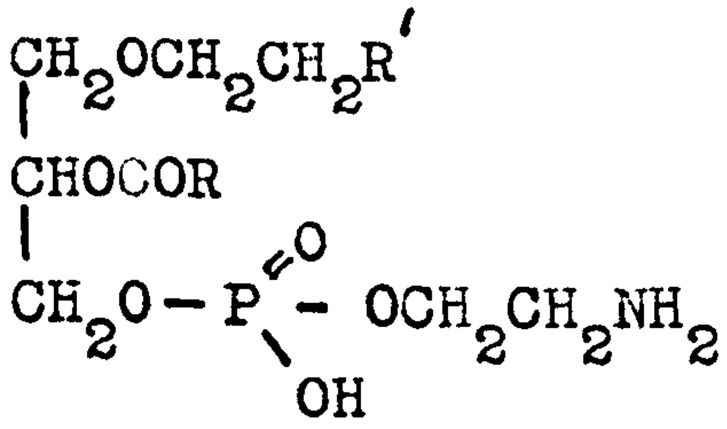
Another group of interesting compounds are the phosphatides of Batyl and Chimyl alcohols. These alcohols

have the following basic structure:-



These compounds are known by the trivial names of batyl, chimyl and selachyl alcohols where the radical R is octadecyl, hexadecyl and oleyl respectively. They have been discovered in the liver oils of various marine animals e.g. by Tsujimoto and Toyama ( Chem. Umschau 29 27 (1922); 31 13, 61, 153 (1924); Chem. Zent. 1 878 (1922)).

Klenk and Debuch (Z. Phys. Chem. 296 179 (1954)) isolated a cephalin from brain which they claimed by degradative studies to have the following structure:-



This is a cephalin of an alcohol such as batyl alcohol.

Much work on the synthesis of phosphatides of batyl and chimyl alcohols was carried out by Baylis (Ph.D. thesis, Bristol. 1956 ).

Analogues of the lecithins and cephalins in which the

alcohol is ethylene glycol, or a long chain aliphatic alcohol instead of glycerol have been prepared by various workers e.g.

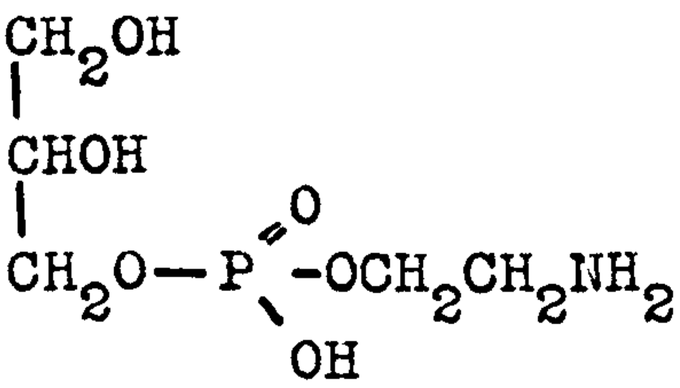
Glycollecithins. Baer and Kates. JACS. 72 942 (1950)

Glycol cephalins. Baylis, Bevan and Malkin. (Report on Biochemical problems of lipids. Ghent (1955) Butterworths)

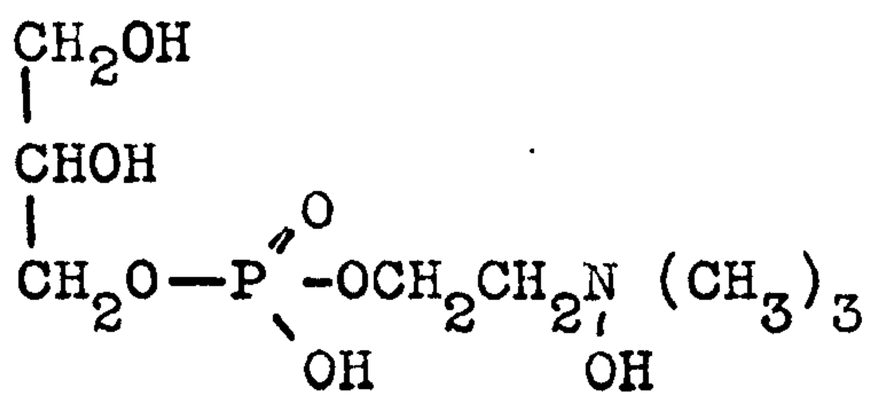
Alkyl analogues of cephalin. Baylis and Malkin (In preparation)

Finally, mention must be made of Glyceryl phosphoryl choline and Glyceryl phosphoryl ethanolamine.

The structure of these compounds is as follows.



Glyceryl Phosphoryl Ethanolamine (GPE)



Glyceryl phosphoryl choline (GPC)

These compounds are produced by the enzymatic action of lecithinases in plants.

Both of the above have been synthesised e.g.

GPC. Baer and Kates JACS. 70 1394 (1948)

GPE. Baer and Stancer JACS 75 4510 (1953).

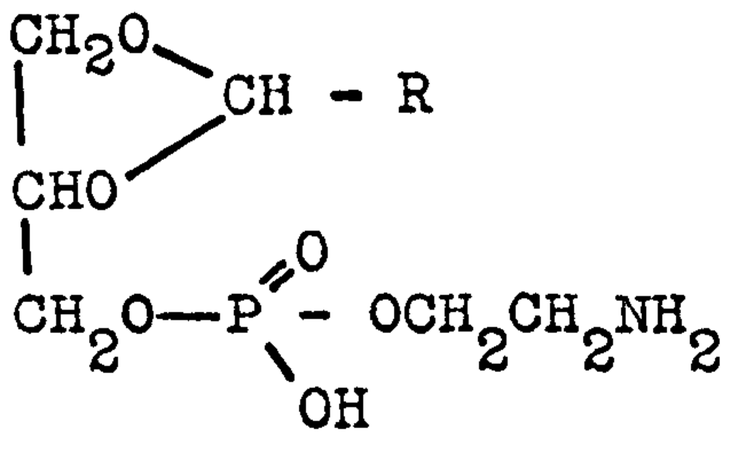
Glyceryl phosphoryl choline is of interest as an intermediate in the synthesis of lecithin, as described in a very recent paper, the GPC being directly acylated to give the corresponding lecithin. (Tattrie and McArthur. Canad. J. Bioch. Physiol. 35 1165 (1957) )

Glyceryl phosphoryl ethanolamine has been isolated from the water soluble nitrogenous constituents of liver by Campbell and Work (Biochem. J. 50 449 (1952) ).

b) Acetal Phosphatides.

The acetal phosphatides, or plasmalogens, were isolated for the first time as a distinct entity by Feulgen and Bersin (Zeit. Physiol. Chem. 260 217 (1939) ), from beef muscle phosphatides. Earlier workers had suspected the presence of an aldehyde containing substance in the cytoplasm of cells. (Feulgen and Rossenbeck Zeit. Physiol. Chem. 135 230 (1924) ).

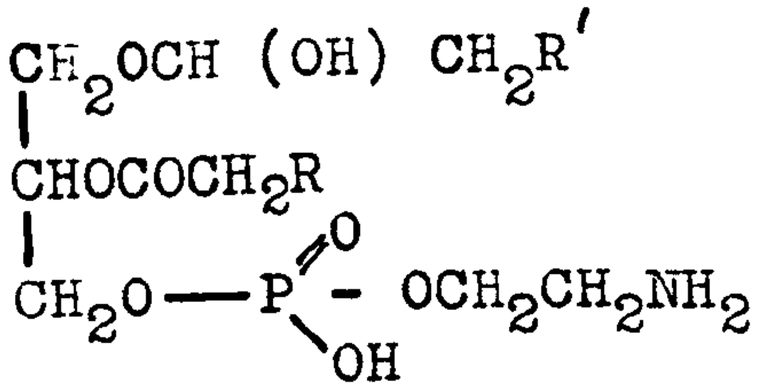
In 1944 Klenk and Schumann isolated plasmalogen from human brain and proposed the following acetal formula:- (Klenk and Schumann. Zeit Phys. Chem. 281 25 (1944))



R - long chain fatty acid residue.

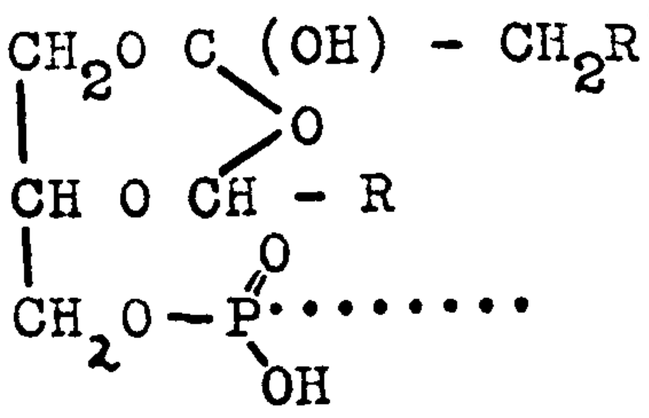
This was supported by Thannhauser, Boncoddo and Schmidt (J.B.C. 188 417 (1951)).

In recent years doubt has been cast on the acetal formulation of plasmalogen and three alternative configurations have been suggested:-



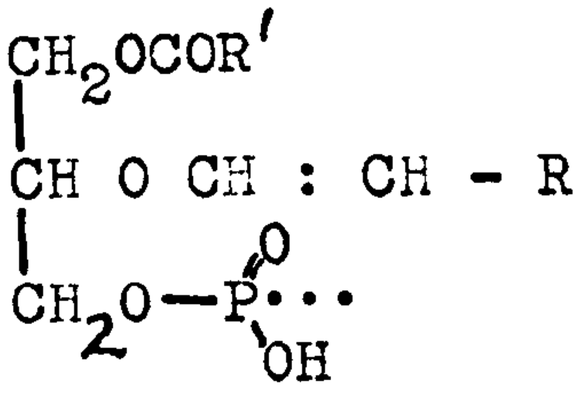
- "Hemiacetal" formula.

Klenk Zeit. Physiol. Chem. 288 98 (1951)



- "Hemiacetal" ortho ester formula.

Baer JACS. 75 4510 (1953)



- Unsaturated ether formula.

Rapport et al. J.B.C. 225 851 (1957)

The decision between these formulae must still be considered an open question although further evidence for

the unsaturated ether formula has recently been adduced. (Gray. Biochem. J. 67 26P (1957) )

Plasmalogens containing choline and serine have also been described e.g. Choline plasmalogens, Klenk and Debuch. Zeit. Phys. Chem. 299 66 (1955).

Rapport et al. J.B.C. 217 199 (1955); ibid 225 851 (1957)

and Acetal phosphatidyl serine:-

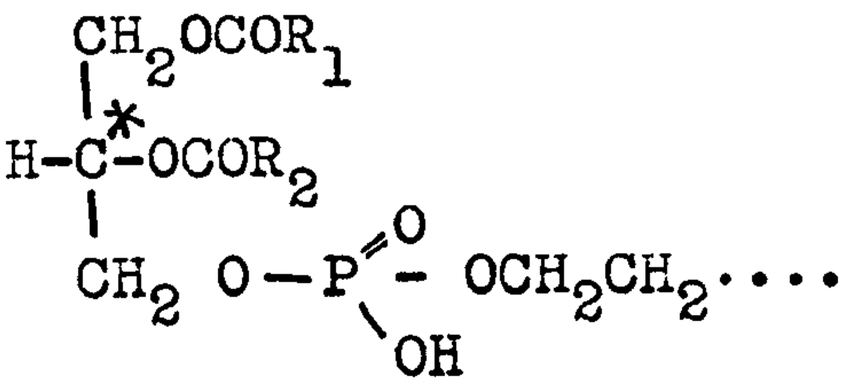
Klenk. Z Phys. Chem. 288 98 (1951).

Ansell and Norman. Bioch. J. 59 IX (1955)

Note on naturally occurring β glycerophosphatides.

The phosphate group in the glycerophosphatides can be attached either at an α or β position in the glycerol molecule. Examples of both α and β glycerophosphatides of unequivocal structure have been synthesised by several workers. e.g. Bevan and Malkin: α and β diacyl cephalins. J. 2667 (1951).

Regarding the naturally occurring glycerophosphatides, the existence of the α isomers has been established by the observation that the compounds are optically active, and hence possess an asymmetrical carbon atom. This can only be so if they are formulated as follows:-



* - ASYMMETRICAL CARBON ATOM.

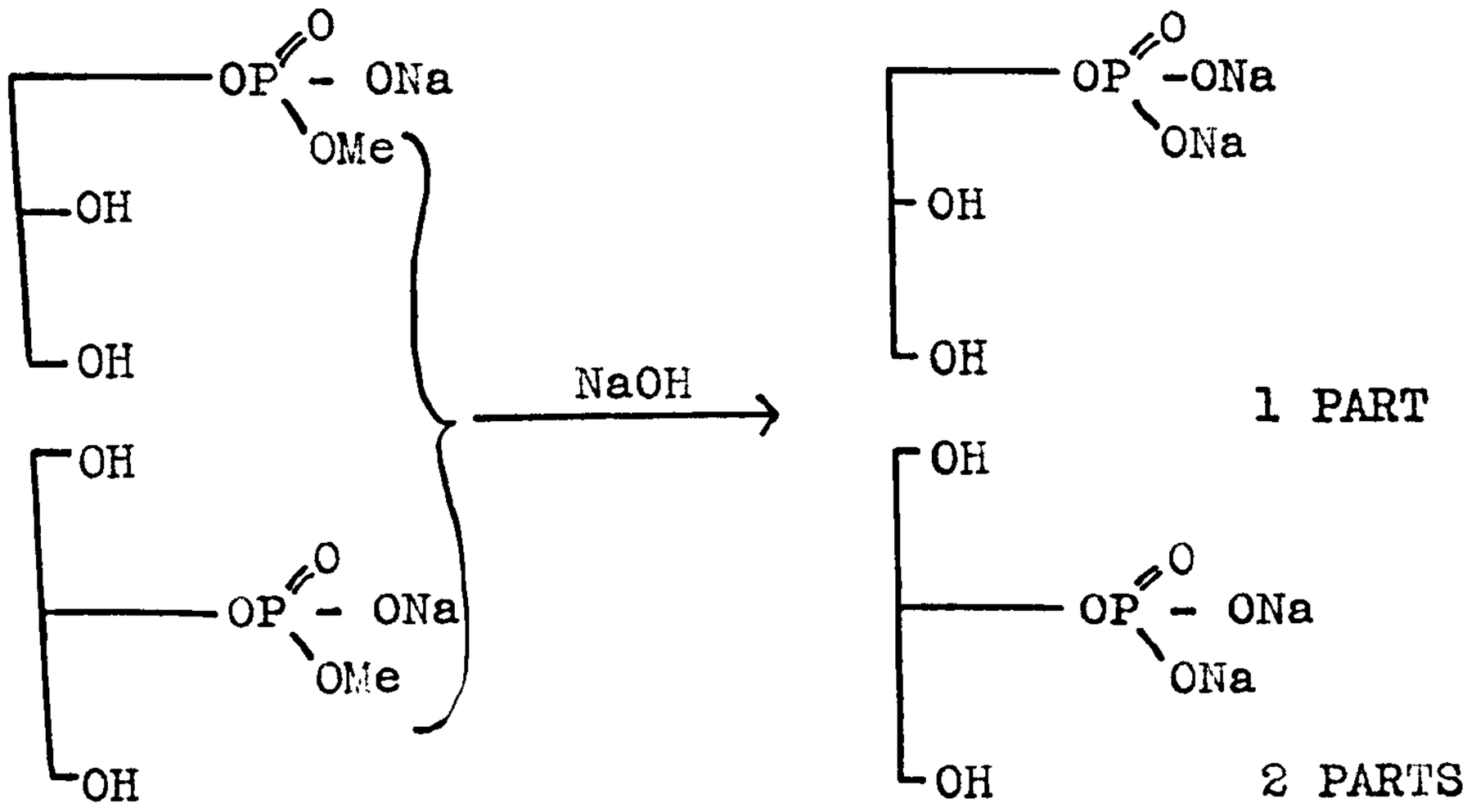
∝ DIACYL GLYCEROPHOSPHATIDE.

(Baer and Kates J.B.C. 200 251 (1953))

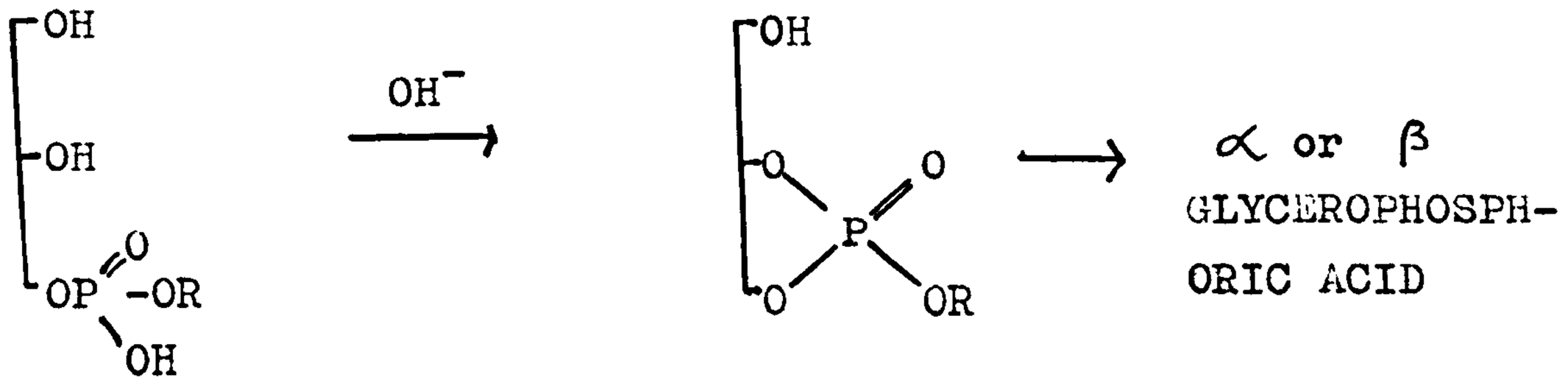
This was confirmed by the enzymatic work of Maguire and Long. (Biochem. J. 57 223 (1954))

The β glycerophosphatides are symmetrical compounds, and are thus optically inactive. The first evidence in support of the existence of β glycerophosphatides was given by Karrer and Saloman (Helv. Chim. Acta. 9 3 (1926)) who isolated β glycerophosphoric acid from the hydrolysis products of natural lecithin.

Doubt was cast on this by Bailly and Gaumé (Bull. Soc. Chim. 2 354 (1935)), who found that alkaline hydrolysis of either ∝ or β sodium methyl glycerophosphate always yielded a mixture of ∝ and β disodium glycerophosphates in the proportion of 1 : 2.




Verkade (Rec. Trav. Chim 59 886 (1940)) suggested that alkaline and acid hydrolysis of glycerophosphate esters proceeded via the formation of a cyclic intermediate:-



This was supported by the work of Chargaff (J.B.C. 144 455 (1942)) who showed the migration was completely intramolecular, using radioactive phosphorus ($P^{32}$) as a tracer.

Baer and Kates (J.B.C. 175 79 (1948) ; ibid 185 615 (1950)) discovered that acid or alkaline hydrolysis of $\alpha$ lecithins yielded a mixture of $\alpha$ and $\beta$ glycerophosphoric acids. The have also shown that the X-ray patterns of synthetic L $\alpha$ dipalmitoyl lecithins are identical with those of natural compounds. (JACS 70 1394 (1948)). Baer considers this proof that $\alpha$ glycerophosphatides are the only isomers occurring naturally.

The work of Baer and Kates was confirmed by Poole (Ph.D. Thesis, Bristol 1951) in his work on the hydrolysis products of hydrogenated natural lecithin, which he compared with those of synthetic compounds.

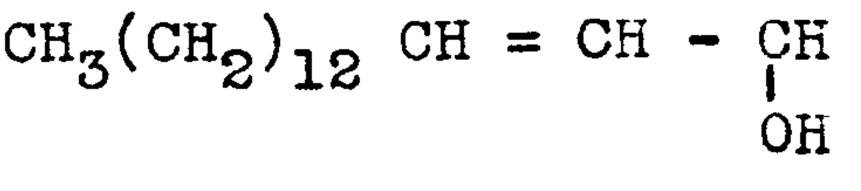
Uhlenbroek and Verkade (Rec. Trav. Chim. 72 550 (1953)) discovered that treatment of a number of $\alpha$ and $\beta$ glycerophosphatidic acids with boiling N/10 ethanolic sodium hydroxide yielded the corresponding $\alpha$ or $\beta$ glycerophosphoric acid respectively. These workers do not share the views of Baer and Kates regarding the non-existence of $\beta$ glycerophosphatides in nature.

In the light of the above evidence, the question must

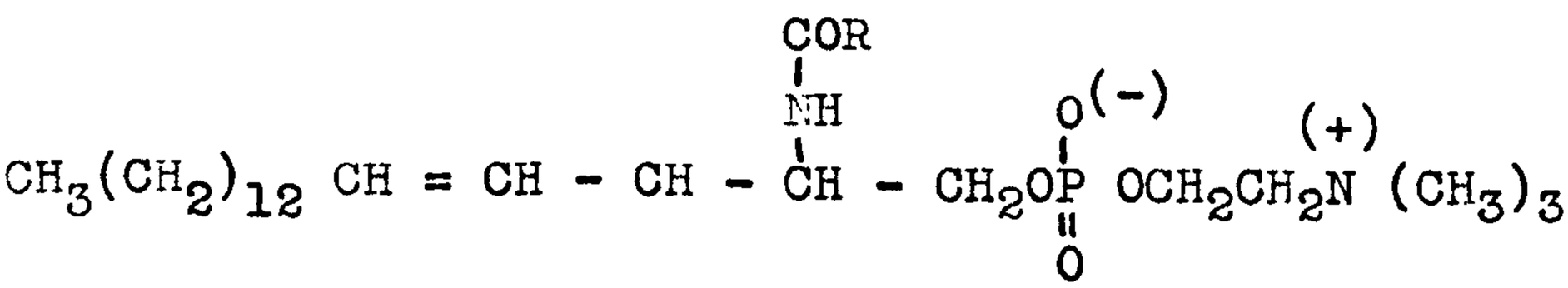
be regarded as still open. Further work on natural glycerophosphatides would be desirable, especially that aimed at the isolation of pure substances of definite composition and structure.

Sphingolipids.

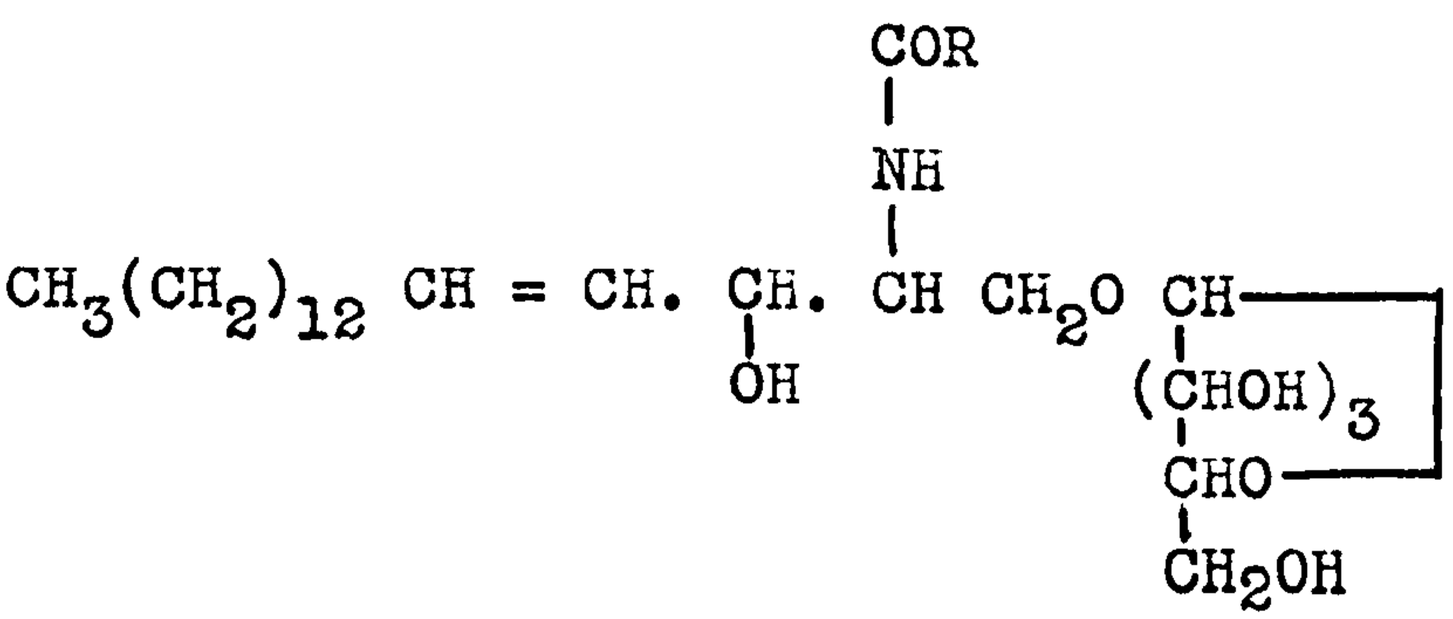
The sphingolipids differ from the glycerophosphatides in that they are derived from the base sphingosine, which has the following structure:-



There are two members of this class of lipid, namely sphingomyelin and cerebroside.



SPHINGOMYELIN.



CEREBROSIDES.

R is usually a lignoceryl group $C_{23}H_{47}CO$ -

The cerebrosides contain no phosphorus, but they are linked to a galactose residue.

The above compounds exist only in the animal kingdoms as far as is known at the present time, although a compound related to Sphingosine was isolated from corn and soya bean inositol phospholipids by Carter et al (J.B.C. 206 613 (1954)). This compound they called phytosphingosine. The best sources of sphingolipids are the brain and spinal cord, and for this reason it is thought that they play a part in the transmission of brain messages.

## Review of the literature on the extraction of phosphatides from natural sources.

### Nomenclature.

A rational system for naming the glycerophosphatides was proposed by Folch (J.B.C. 174 439 (1948)), based on the parent phosphatidic acid. Thus cephalin became phosphatidyl ethanolamine and lecithin phosphatidyl choline.

Before this, the nomenclature had been very arbitrary, depending on the solubility of the substance in alcohol. The alcohol soluble portion was termed lecithin, while the insoluble portion "cephalin fraction". The latter was actually a mixture of at least three classes of compounds, the cephalins, phosphatidyl serines and phosphoinositides.

Isolation of phosphatides from natural sources.

Lecithins

The lecithins were first isolated rather more than a hundred years ago by Gobley (J. pharm. chim. 9 1, 81, 161 (1846) ; ibid 11 409 (1847) ; 12 1 (1847)). The presence of basic nitrogen was demonstrated by Strecker (Ann. Chem. Pharm. 148 77 (1868)) by the formation of the chloroplatinate, and he postulated the formula which is now accepted for these compounds.

Thudicum ("Die Chemische Konstitution des Gehirns des Menschen und die Tiere" - Tubingen, Franz Pietzcker 1901) in his classic treatise on the phosphatides isolated lecithin from brain and confirmed its structure.

An important advance in the purification of lecithin was made by McLean (X. Physiol. Chem. 59 223 (1909A)), who was the first worker to obtain the cadmium chloride complex of natural lecithin as a precipitate. The cephalin phosphatides remained in the filtrate. He further observed that extraction of the cadmium chloride complex with ether removed other impurities, and hence obtained a relatively pure sample of lecithin (J. Path. Bact. 18 490 (1914a); C.A. 9 1341 (1915) : Bioch. J. 9 351 (1915) ). Levene and Rolf refined this method for the preparation of lecithin from natural sources (J.B.C. 46 193 (1921) ; ibid 72 587 (1927) ), and brought it to such a state of efficiency that

it was possible to prepare lecithin from natural sources in as little as 24 hours. The preparation of crude cadmium chloride complex followed by trituration with ether and subsequent regeneration of the lecithin was the basis of the method.

Further improvements in the purification of isolated lecithin (Maltaner JACS. 52 1718 (1930)) and of the cadmium chloride complex (Pangborn J.B.C. 137 545 (1941) ; ibid 188 471 (1951) ) have been made. Escher ( Helv. Chim. Acta. 8 686 (1925) ) and Sinclair (Canad. J. Research. 12 777 (1949) ) have described the preparation of pure lecithin by means of a fractional crystallisation process. In recent years Lea, Rhodes and Stoll (Bioch. J. 60 353 (1955)) have isolated pure phosphatidyl choline and phosphatidyl ethanolamine from egg phospholipid by chromatographing in methanol/chloroform on silicic acid.

Cephalins.

The isolation and elucidation of the structure of cephalin was slower than that of lecithin owing to two difficulties;

a) The complex composition of"cephalin fraction"

b) The lack of insoluble derivatives of cephalin.

Thudicum first isolated it from brain tissue in 1884 ("A Treatise on the Chemical Constitution of the Brain" London

Baillere, Tindall and Cox. 1884).

Trier, in a series of papers published between 1911 - 1913 described the discovery of ethanolamine in phosphatides from the bean "Phaseolus vulgaris", and he identified it by its insoluble gold chloride complex. He also observed that Cadmium chloride in ethanol was an efficient way of separating lecithins and cephalins. (Z. Phys. Chem. 73 383 (1911) ; ibid 76 496 (1912) ; ibid 86 1, 141, 153 (1913)).

Levene and West carried out much work on these compounds the culmination of which was the hydrogenation of natural cephalin to yield a saturated compound which gave correct analytical results. Levene and West (J.B.C. 24 41 (1916a); ibid 25 517 (1916b) ; ibid 35 285 (1918)).

Rudy and Page (Z. Phys. Chem. 193 251 (1930)) drew attention the solubility of cephalin in alcohol, since up to this time the separation of " cephalin fraction" had depended on its insolubility in alcohol. In 1932 two Japanese workers, Nishimoto and Suziki ( Proc. Imp. Acad. (Tokyo) 8 424 (1932)) claimed to have isolated $\alpha$ distearoyl cephalin from human brain, by bromination of their crude product and separation into three fractions by solubility differences. A m. pt. of 175° C was quoted.

The first worker to separate "cephalin fraction" into

its constituents was Folch, and he did this by dissolving it in chloroform and adding alcohol to the solution. The phosphoglyceroinositide was precipitated first, being the least soluble in alcohol, followed by phosphatidyl serine, while the phosphatidyl ethanolamine remained in solution. Folch (J.B.C. 146 35, (1942a)).

Although this was a great advance at the time, Lovern (Bioch. J. 51 464 (1952)) has shown by counter current technique applied to ox brain phosphatides that Folch's phosphatidyl ethanolamine was contaminated with phosphatidyl serine.

The recent work of Lea, Rhodes and Stoll (loc. cit.) on the chromatographic purification of phosphatidyl ethanolamine suggests that future application of this method to the preparation of individual cephalins from natural sources may be possible.

Phosphatidyl serine.

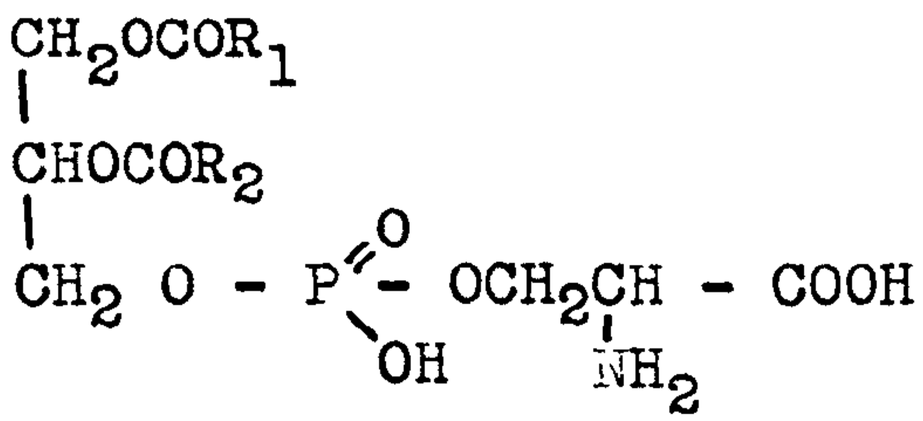
Several early workers suspected the presence of an amino acid in "cephalin fraction" obtained from natural sources (McArthur JACS 36 2397 (1914) ; Darrah and McArthur (JACS 38 922 (1916); McArthur, Norbury and Karr; JACS 39 768 (1917) ; Thierfelder and Schulze; Z. Phys. Chem. 96 296 (1915/16) ). This view was confirmed by Christensen and Hastings (J.B.C. 136 387 (1940) ) in their work on the electrophoresis and

titration of egg yolk "cephalin fraction". A divergence from the accepted formula was indicated, and it was found that 0.6 equivalent of alkali metal were bound to each mole of "cephalin".

The work of Folch and Schneider (J.B.C. 137 51 (1941) proved the presence of serine in ox brain "cephalin fraction". It was found that this supposed serine reacted with ninhydrin liberating carbon dioxide, a reaction which characterises $\alpha$ amino acids, and a glycolic aldehyde was obtained from this, which was identified as its dimedone derivative or phenylosazone. Periodate oxidation yielded ammonia, indicating that a hydroxyl group adjacent to the amino group was present. This evidence proved that the amino acid was serine.

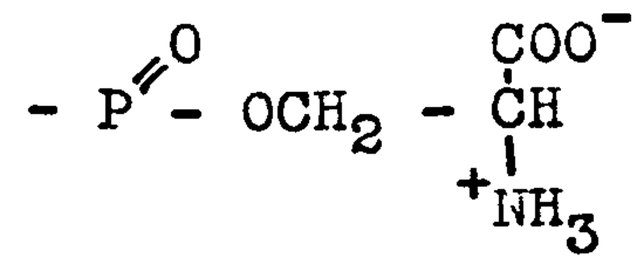
Folch (J.B.C. 139 973 (1941a) ; ibid 146 35 (1942a)) isolated pure phosphatidyl serine, and hence L serine, in the form of its sodium or potassium salt by fractional precipitation with alcohol from a chloroform solution of "cephalin fraction".

In a later paper (Folch J.B.C. 174 439 (1948)) he demonstrated that the amino and carboxyl groups were free and postulated the following formula, now universally accepted.



The alkali metal predominately associated with phosphatidyl serine is potassium.

Recently however, Garvin and Karnovsky (Fed. Proc. 13 215 (1954)) have shown by electomeric titration in a system of 99% ethoxyethanol, 1% water and 0.001 M potassium chloride that partially unsaturated ox brain phosphatidyl serine has the carboxyl group mainly in the anionic form, and the ~~o~~ ∝ amino group entirely in amine salt form,viz:-



This is probably also true of saturated phosphatidyl serine.

An interesting suggestion was made by Koch and Pike (J. Pharmacol. 2 245 (1910); C.A. 5 2263 (1911)) regarding the alkali metal salts of phosphatidyl serine- it was that these phosphatides contribute to the maintainance of electronic equilibria in nerve cells.

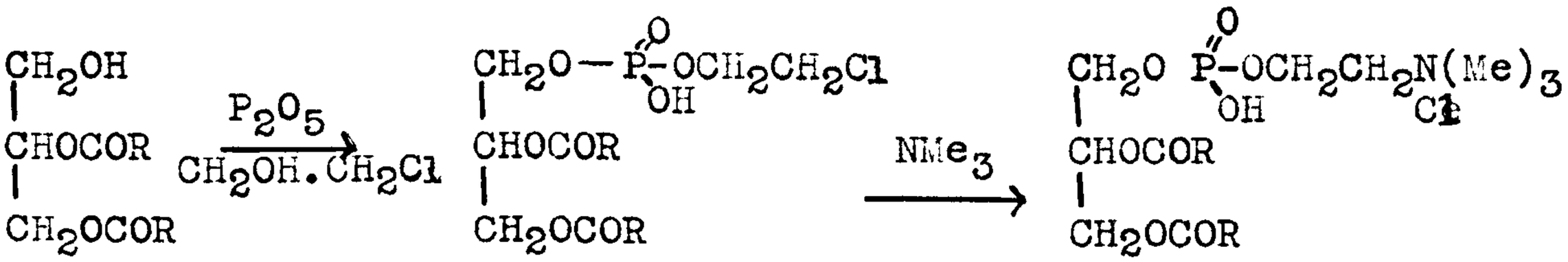
Serine itself was first isolated from sericin hydrolysate by Cramer (J. Prakt. Chem. 96 76 (1865)). This gave the racemic form, as did the method of Daft and Coghill (J.B.C. 90 341 (1931)). The L form of serine is the one found in

all naturally occurring compounds, and Artom, Fishnan and Morehead (Proc. Soc. Exptl. Biol. Med. 60 284 (1945)) have shown that the D form is toxic.

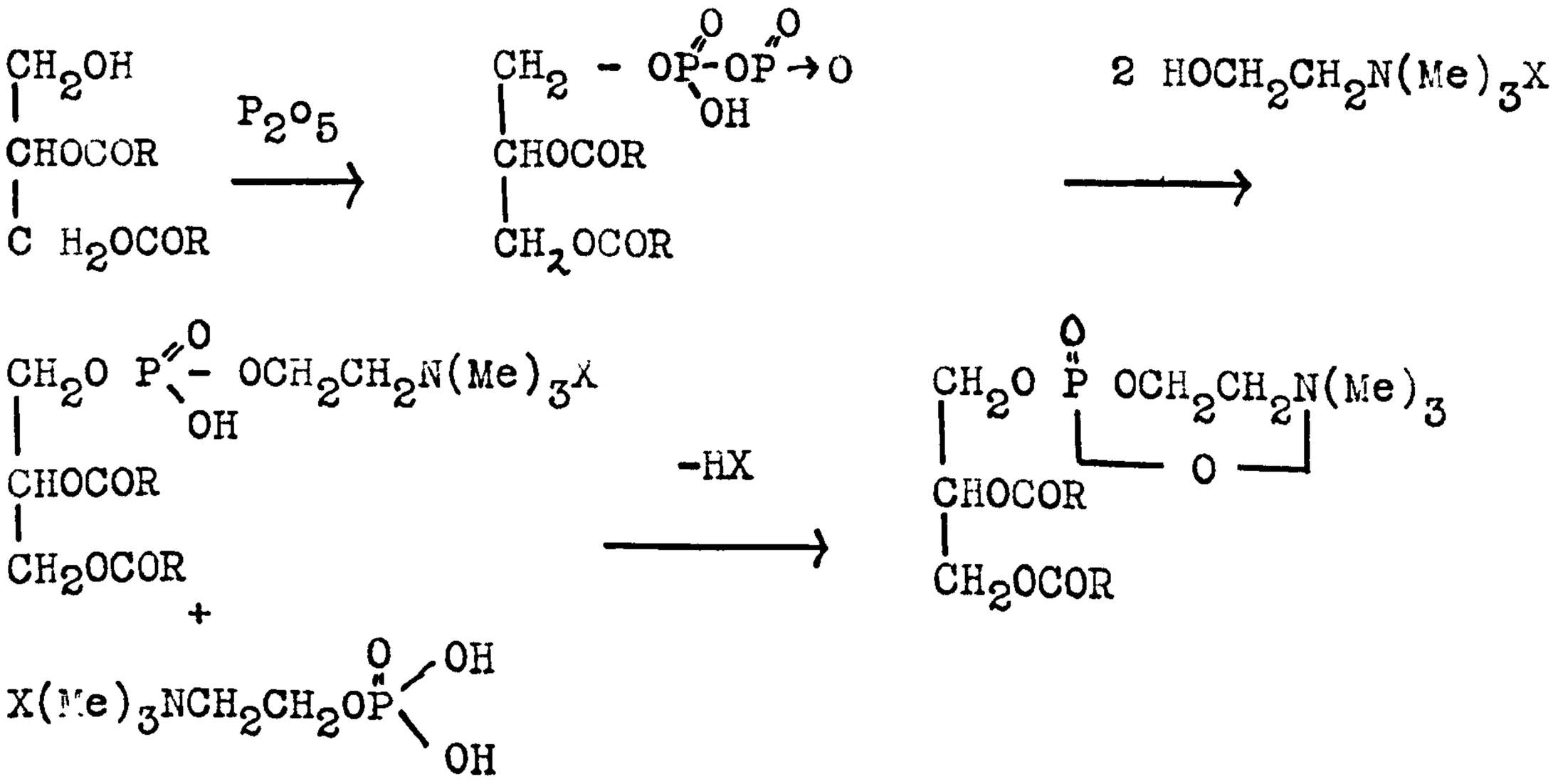
Review of the literature on synthesis of glycerophosphatides.

Lecithins.

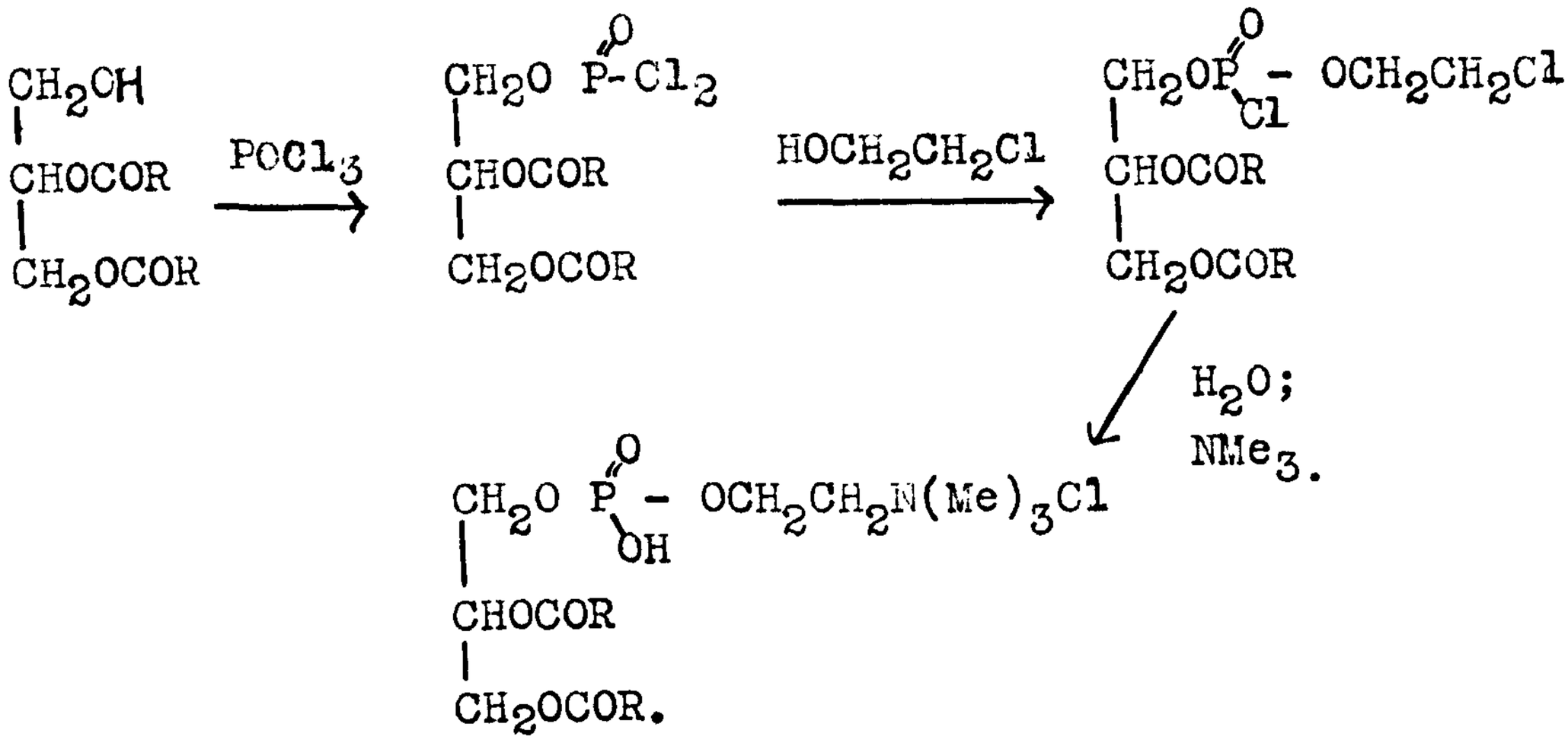
The first reported synthesis of a lecithin was that of Grün and Kade (Ber. 45 3367 (1912)) who used the following reaction scheme.



In 1926 Grün and Limpacher modified this method by the use of choline bicarbonate or acetate (Ber 59 1350 (1926)).



In a third synthesis reported in 1936 phosphorus oxychloride was used as the phosphorylating agent. viz:-

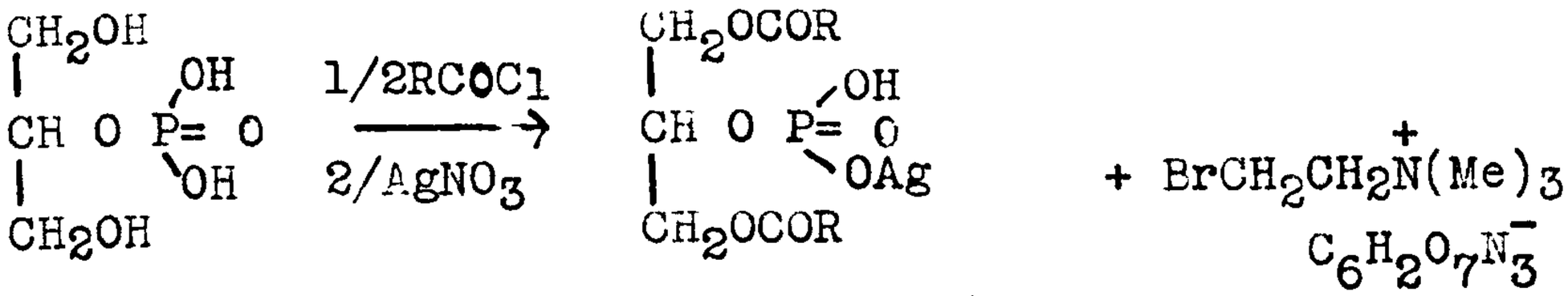


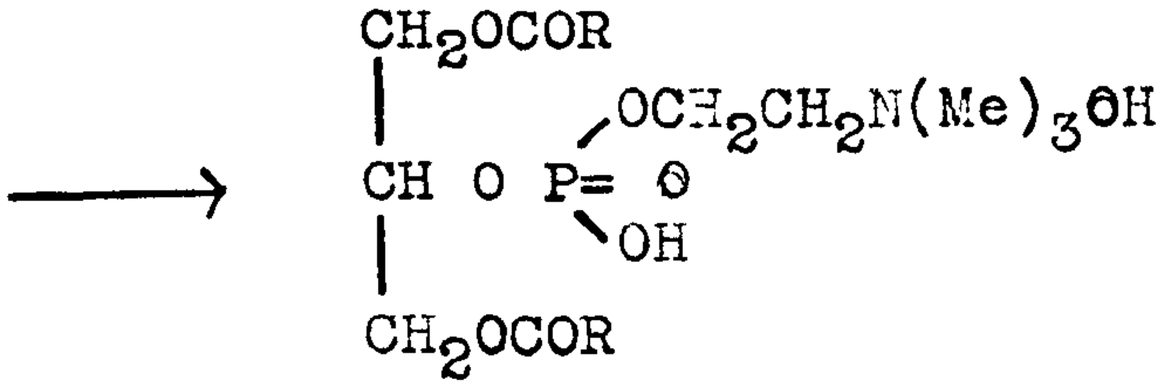

(Grün. Chimie und Technologie der Fette und Fetteprodukte Schonfeld Springer 485 (1936)).

It is unlikely that the products obtained in any of the above methods were pure lecithins. Probably choline salts of phosphatidic acids were present, since $P_2O_5$ and $POCl_3$ are not ideal phosphorylating agents for lecithin synthesis. The diglycerides used by Grun were probably 1 : 3 diglycerides since at the time no satisfactory method for the preparation of 1 : 2 diglycerides had been found.

Kabashima (Ber. 71 76 (1938) ; ibid 71 1071 (1938a)) claimed to have prepared lecithins by the acylation of glycerophosphoric acid with palmitoyl chloride, followed by the reaction of the monosilver salt of the resulting phospha-

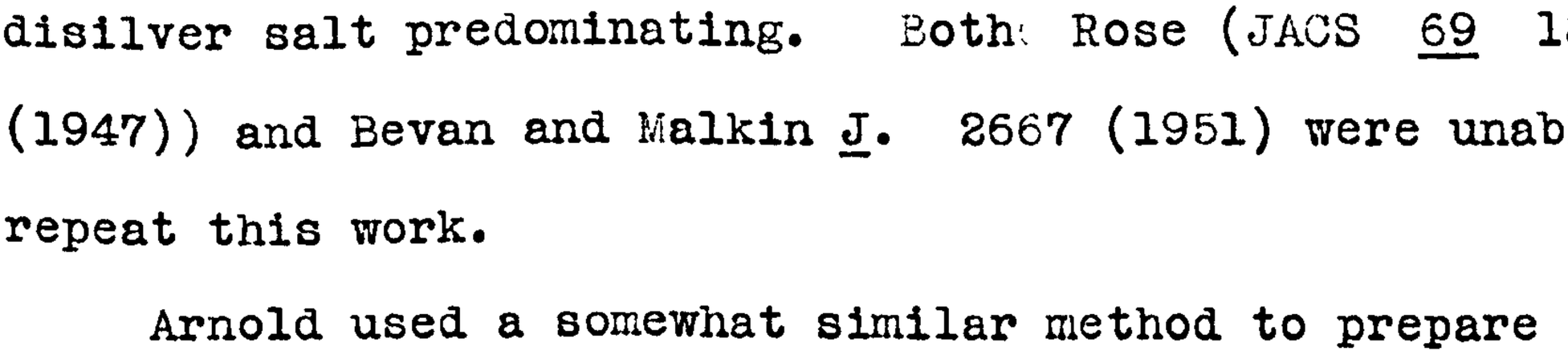
-tidic acid with bromocholine picrate. viz:-





Difficulties arise with the acylation of the β glycerophosphoric acid, and the formation of the monosilver salt, the disilver salt predominating. Both Rose (JACS 69 1384 (1947)) and Bevan and Malkin J. 2667 (1951) were unable to repeat this work.

Arnold used a somewhat similar method to prepare chaulmoogric and hydnocarpic acid lecithins:- Arnold (Ber. 73 87, 90 (1940)). The disilver salt was prepared by Kabashima's method.

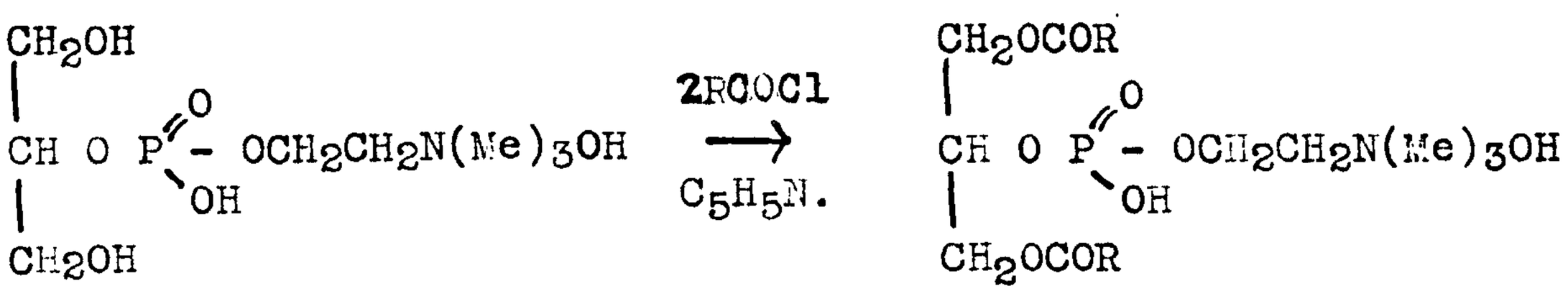


TRIMETHYL β BROMOETHYL

AMMONIUM BROMIDE

Owing to the impurity of the acids used as starting materials in the acylation stage, the products obtained were not pure.

In 1943 a new approach to the problem was described by Obata (Bull. Inst. Phys. Chem. Res. (Tokyo) 22 115 (1943) ; Chem. Abs. 42 522 (1948)). He carried out a direct acylation of glyceryl phosphoryl choline with palmitoyl chloride and pyridine.

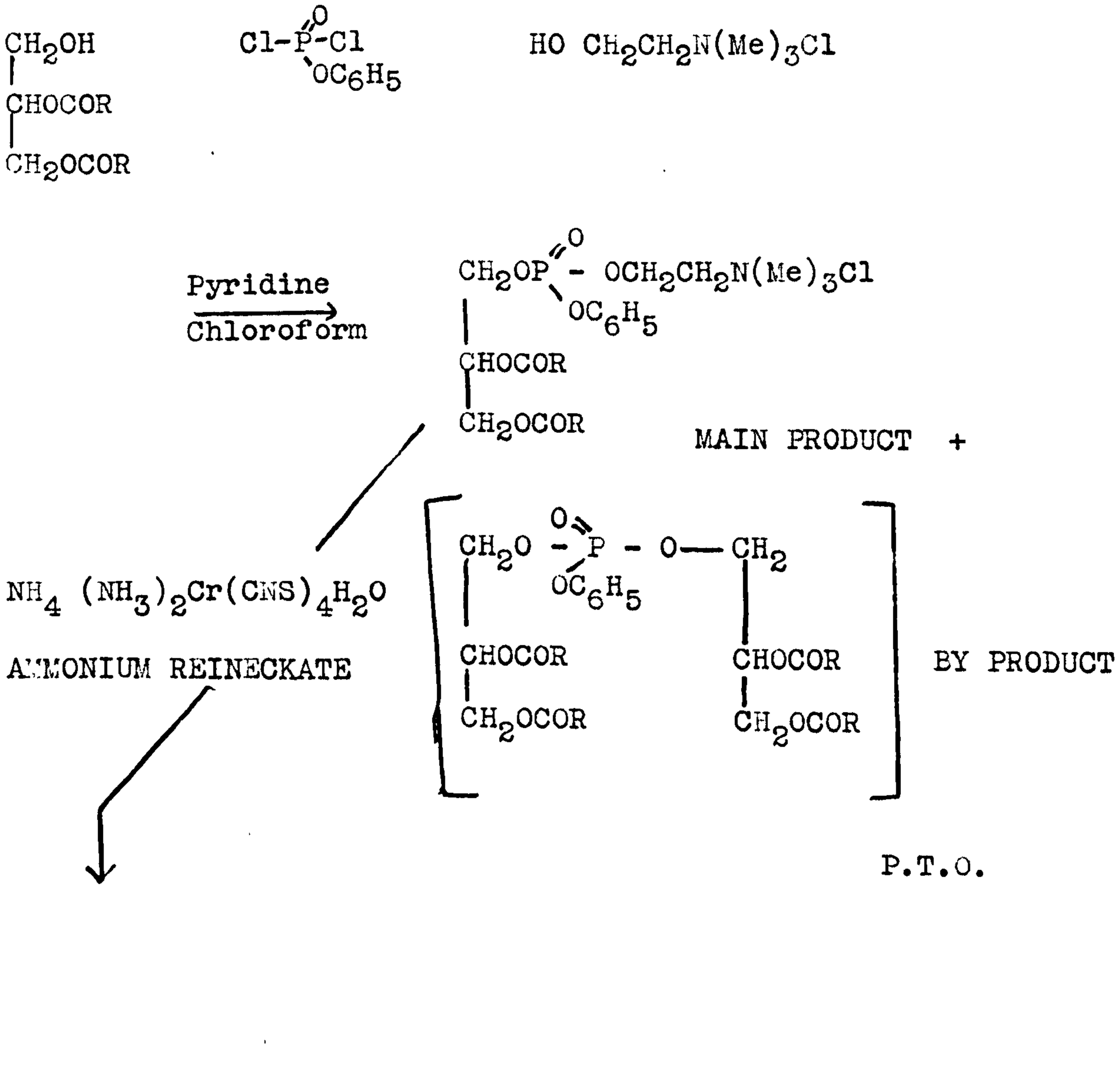



The substance obtained softened at 84° C, became transparent at 160° C and liquefied at 185° C.

It is of interest to note that this method has been revived recently in a method of lecithin synthesis described by Tattrie and McArthur (vide infra).

The first synthesis of an authentic specimen of lecithin was carried out by Baer and Kates (JACS 72 942 (1950)), who prepared a number of L and DL 1,2 DIACYL lecithins. In this preparation a 1,2 diglyceride was phosphorylated with monophenyl phosphoryl dichloride and pyridine, and then treated with choline chloride in the presence of a large excess of

pyridine. The required Diacyl l- glycerylphenyl phosphoryl choline chloride was isolated from the by-product, bis - Diacyl l glyceryl phenyl phosphate, by means of its ethyl acetate soluble reineckate complex. The latter was then converted into the sulphate, the phenyl group removed by catalytic hydrogenolysis, after which the sulphate was converted to the lecithin, which was crystallised from di-isobutyl ketone. The reaction scheme was as follows:-

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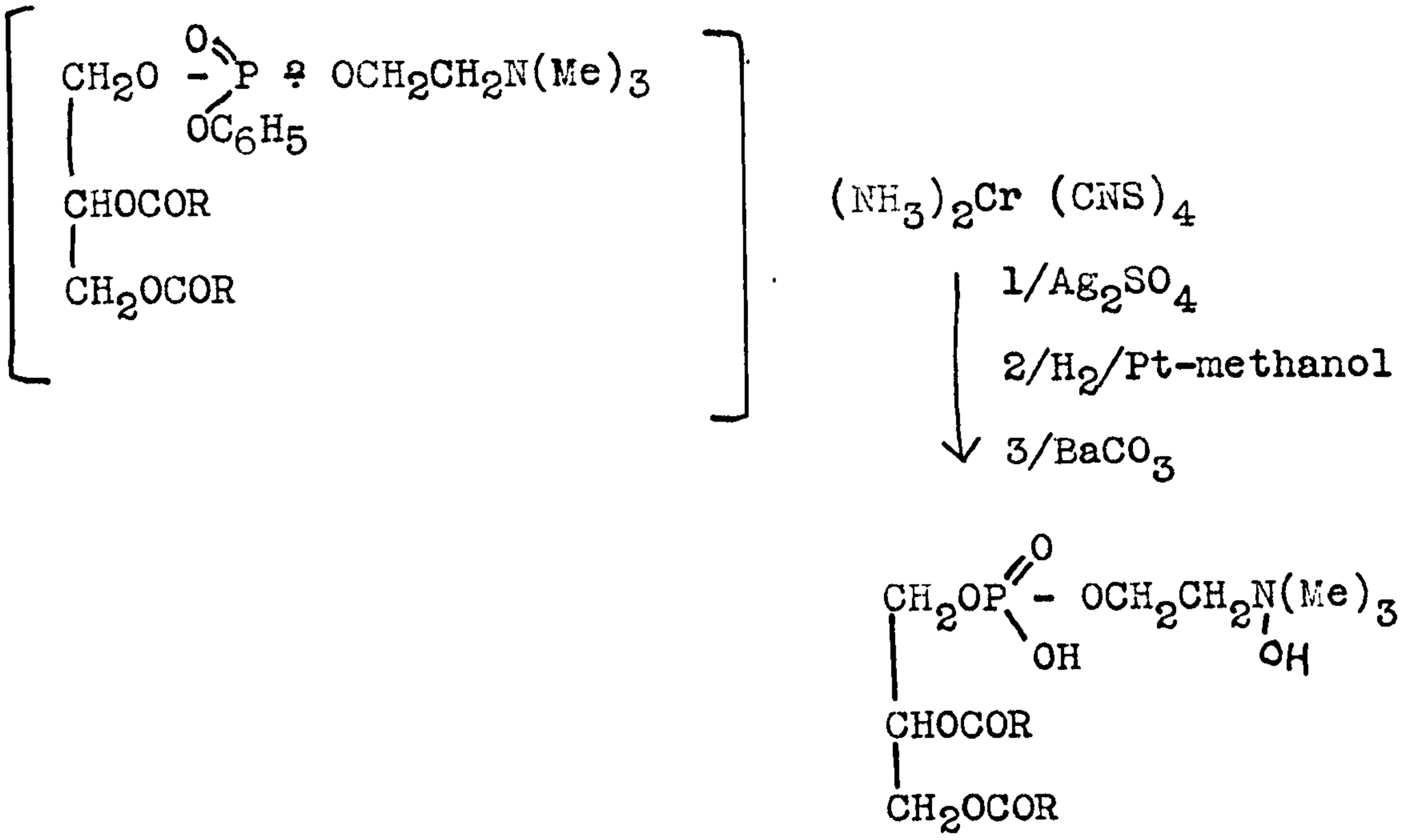



1,2 DIACYL LECITHIN

The synthetic L 1,2 dipalmitoyl lecithin and natural dextrarotatory dipalmitoyl lecithin c.f. Lesuk and Anderson J.B.C. 139 457 (1941); Thannhauser, Benotti and Boncoddo ibid 166 669 (1946); Thannhauser and Boncoddo ibid 172 135 (1948) had the same composition, melting points, solubilities and optical rotations.

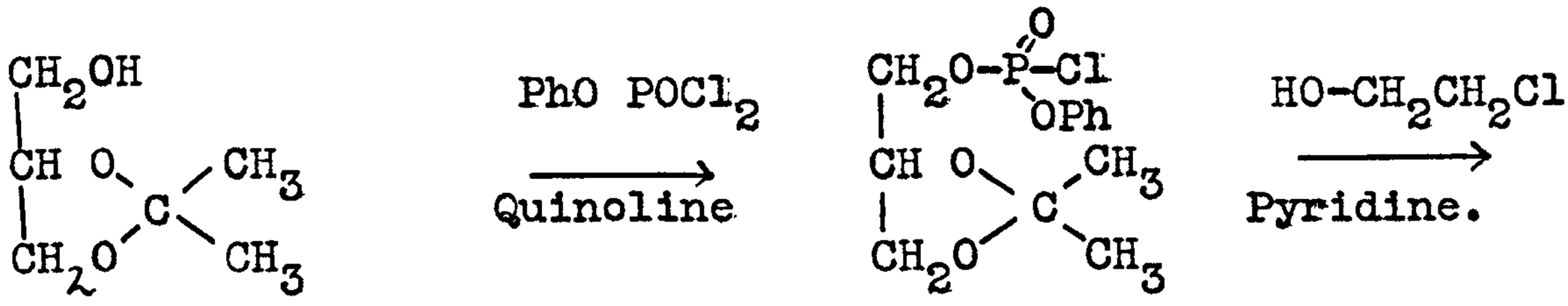
A modification to this method eliminating the reineckate stage described by Baer and Maurukas (J.A.C.S 74 158 (1952)) was used for the preparation of D 1,2 dimyristoyl lecithin (J.B.C. 193 835 (1951)).

Bevan and Malkin (in preparation) have prepared a series

of 1,2 and 1,3 DL lecithins by the method of Baer and Kates except that they used choline iodide instead of the extremely hygroscopic choline chloride.

The synthetic lecithins are white microcrystalline solids with melting points in the region of 230 - 240°C.

The above method of synthesis can only be used for the saturated lecithins since the hydrogenolysis of the protective phenyl group converts any unsaturated acid radicals present to the corresponding saturated compounds. Baer, Buchnea and Newcombe (JACS 78 232 (1956)) have overcome this difficulty by allowing the hydrogenolysis of the phenyl group to precede the acylation of the glyceryl moiety. D acetone glycerol was phosphorylated with monophenyl phosphoryl dichloride and quinoline, and the L ∝ acetone glyceryl phenyl phosphoryl chloride so produced treated with ethylene chlorhydrin and pyridine. Catalytic hydrogenolysis of the phenyl group was then carried out, the acetone group removed by mild acid hydrolysis, and the product isolated as its barium salt. This was acylated with oleyl chloride and pyridine in anhydrous dimethylformamide, and the dioleyl barium salt condensed with trimethylamine in benzene at 60°C for 4 days, a sealed tube being used. The mixture of L 1,2 dioleyl lecithin and oleyllysolecithin was separated on a silicic acid column.




L 1,2 Dioleyl lecithin.

Very recently a method for the preparation of lecithins by the direct acylation of glyceryl phosphoryl choline has been reported by Tattrie and McArthur. (Canad. J. Bioth. Physiol. 35 1165 (1957)). The acylation was carried out in dry chlorform by shaking palmitoyl chloride with GPC at 37° C for 72 hours, subsequently purifying the crude lecithin obtained by chromatographing on silicic acid. An overall yield of 31% was obtained.

Lysolecithins.

These have been synthesised in these laboratories by Baylis, Bevan and Malkin (Report on Biochemical Problems of Lipids. Butterworths Ghent 1955) and Bevan and Malkin (in preparation). The method used was essentially the same as for the diacyl compounds, except that a monoacyl monobenzyl ether of glycerol was used as the starting material and the benzyl protecting group was removed concurrently with the phenyl group by catalytic hydrogenolysis.

Glycollecithins.

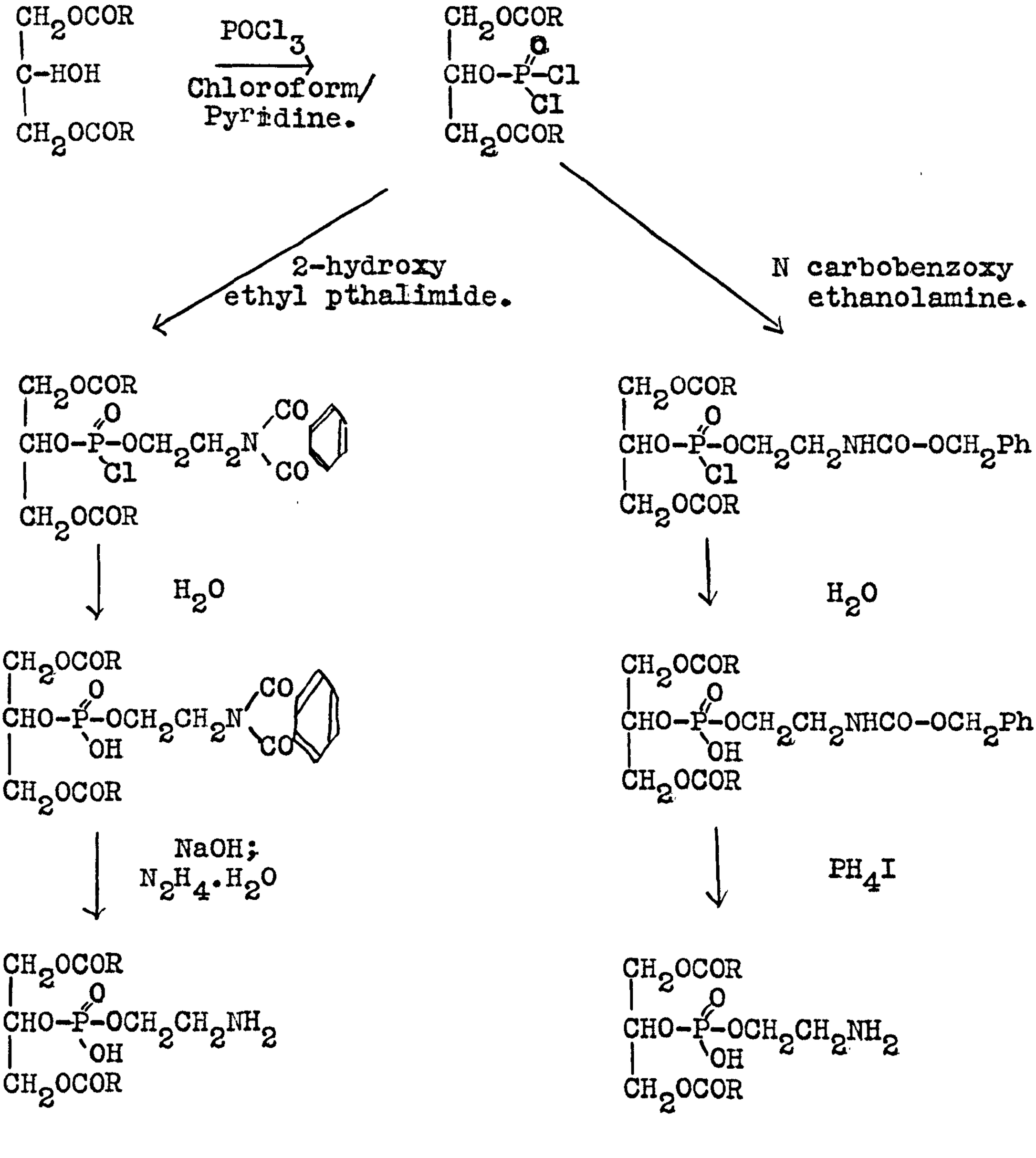
Baer and Kates (JACS 72 942 (1950)) obtained these in 24-30% yield by a similar method to that used for the diacyl glyceryl lecithins. The reduced yield is due to the greater tendency to form bis glycol monophenyl phosphate. The glycol lecithins are much more soluble in water than the glycerol analogues, and like lysolecithins possess haemolytic

properties.

Cephalins.

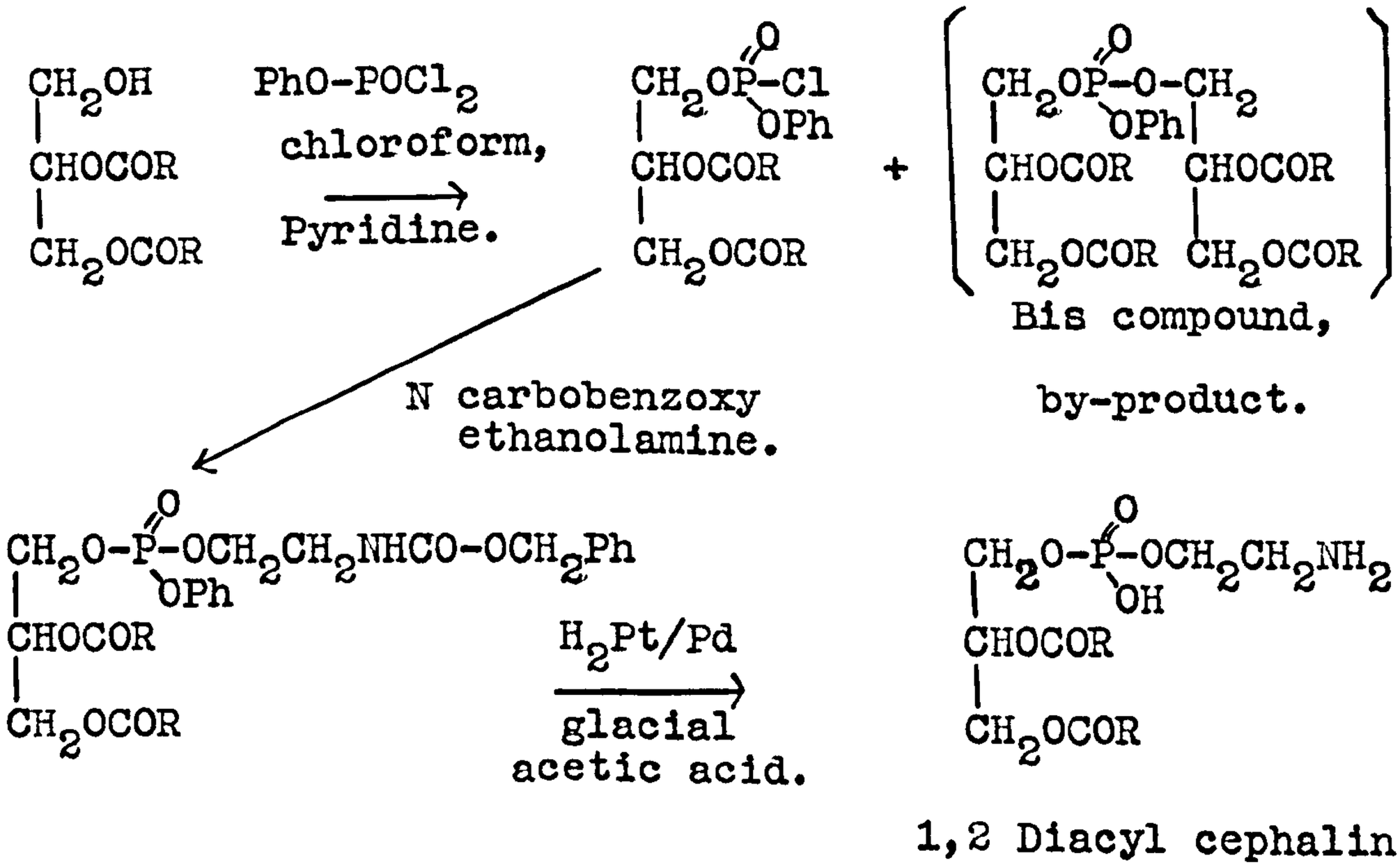
The first synthesis of cephalin was reported by Grun and Limpacher ( Ber 60 147 (1927)) using a method similar to that employed for the lecithins, except that ethanolamine carbonate was used instead of choline bicarbonate. Later, Kabashima (Ber 71 76, 1071 (1938)) also described the synthesis of cephalin by reacting the mono silver salt of a phosphatidic acid with $\beta$ bromoethylamine hydrobromide. The properties reported for the products obtained make it evident that they were impure mixtures of $\alpha$ and $\beta$ cephalins and/or ethanolamine salts of $\alpha$ and $\beta$ phosphatidic acids.

Rose (JACS 69 1384 (1947)) prepared the first authentic specimen of a cephalin by treating a 1,3 diglyceride with phosphorus oxychloride, and then reacting the resulting dichloride with either 2 - hydroxyethyl pthalimide or N carbobenzoxy ethanolamine. After hydrolysis to the intermediate 1,3 diacyl glyceryl 2 pthalyl amino ethyl or N carbobenzoxy ethanolamine phosphates, the protecting groups were removed by hydrazine hydrate or phosphonium iodide respectively. Rose also repeated Kabashima's work, but found that the yield was impracticably small.


POCl3
Chloroform/
Pyridine.
2-hydroxy
ethyl pthalimide.
N carbobenzoxy
ethanolamine.
H2O
H2O
NaOH;
N2H4.H2O
PH4I

Later workers have used Rose's method for the preparation of cephalins (Hunter, Roberts and Kester JACS 70 3244 (1948); Bevan and Malkin J, 2667 (1951)), but they dispensed with the wasteful separation of the intermediate pthalyl and carbobenzoxy compounds.

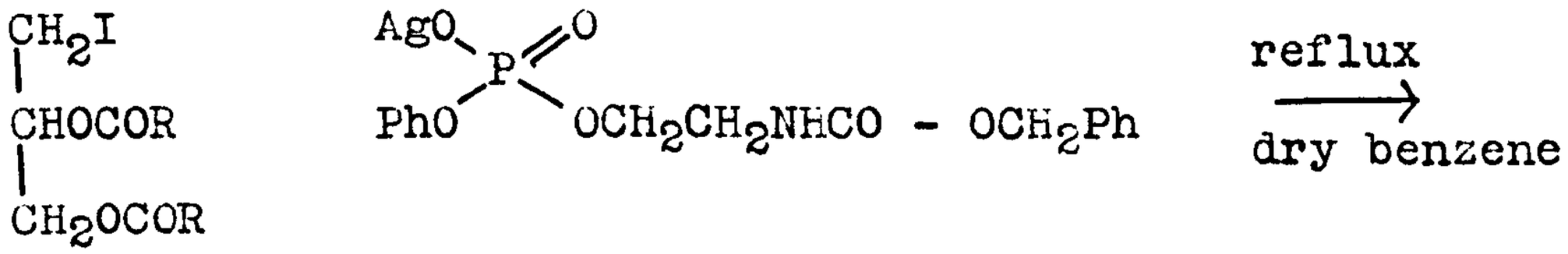
A general synthesis of enantiomeric $\alpha$ cephalins is that due to Baer, Maurukas and Russell (JACS 74 152 (1952)), who used the method to prepare L distearoyl, dipalmitoyl and dimyristoyl cephalins. In principle similar to Rose's method, their scheme utilises monophenyl phosphoryl dichloride as the phosphorylating agent.



The protecting phenyl and carbobenzoxy groups were removed concurrently by catalytic hydrogenolysis using a mixture of Adams' platinum oxide and palladium black catalysts in glacial acetic acid. Rose had been unable to remove the carbobenzoxy group by hydrogenolysis.

All the above syntheses suffer from the disadvantage that it is necessary to start with a 1,2 diglyceride, the preparation of which is rather tedious involving several stages, and also that by-products are formed during the first phosphorylation stage, leading to lowered yields of required product.

In the synthesis described by Baylis, Bevan and Malkin (loc. cit.), these difficulties have been completely overcome. In this method, the readily prepared diacylated glycerol 1-iodide is used as the starting material, being refluxed in anhydrous benzene in the dark with the dry silver salt of phenyl phosphoryl N - carbobenzoxy ethanolamine. The protecting groups are then removed by catalytic hydrogenolysis.

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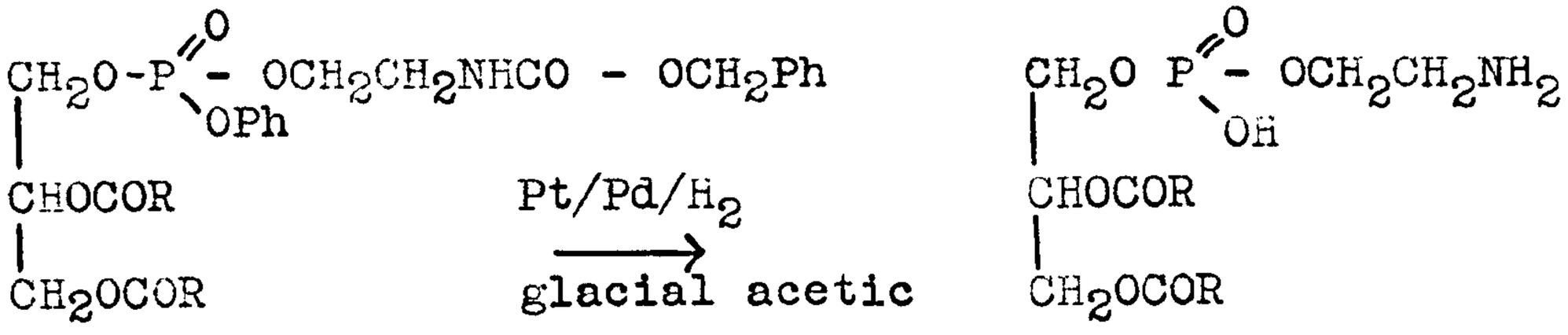
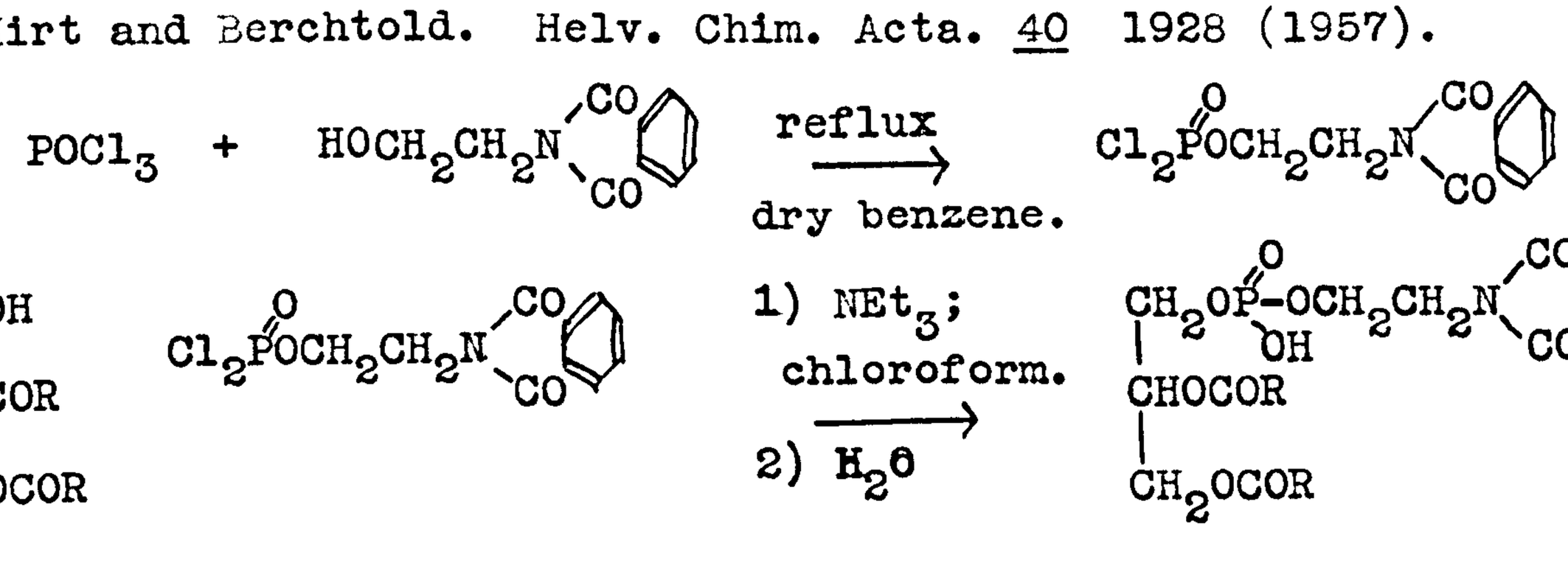
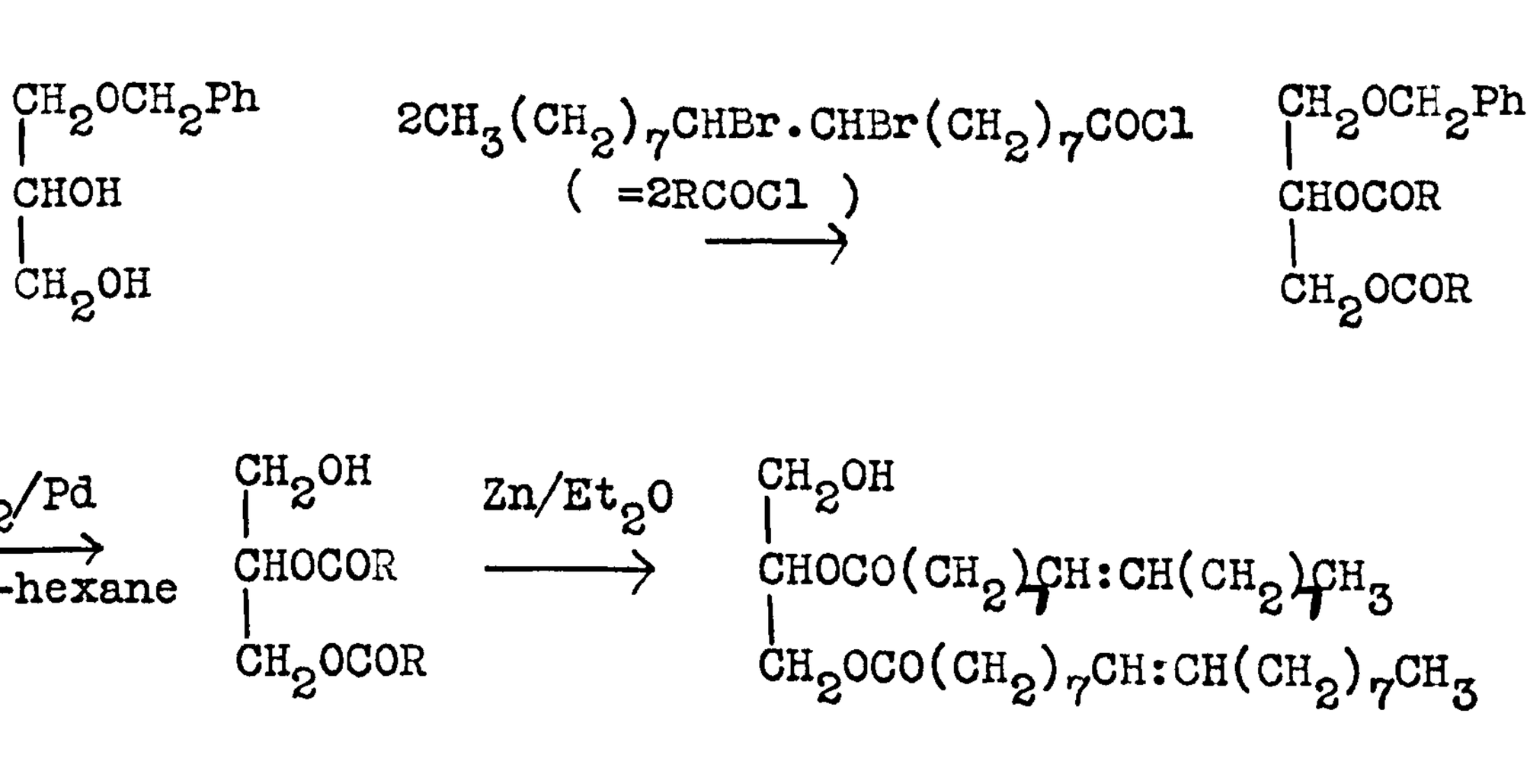


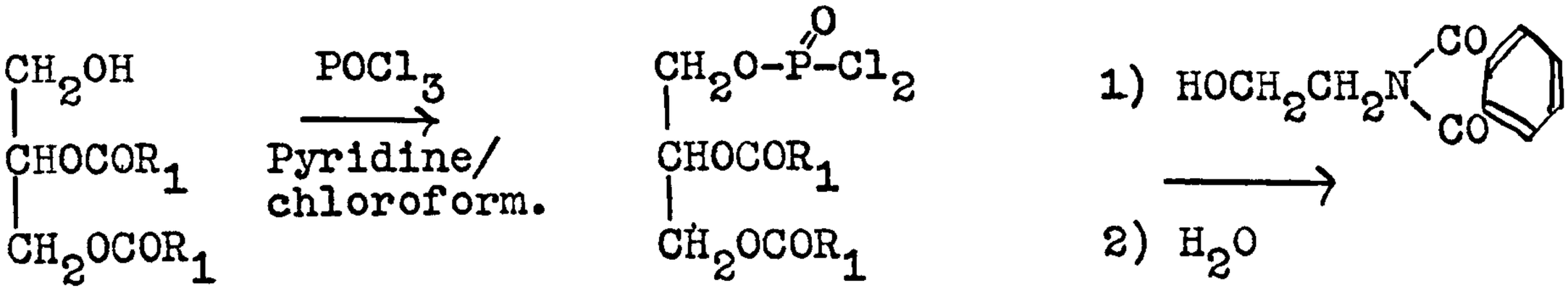

A method very recently published by Hirt and Berchtold is worthy of mention. This is similar to Rose's method except that the 2 - hydroxyethyl pthalimide is reacted with phosphorus oxychloride and the resulting dichloride isolated as a crystalline solid . This is then reacted with the diglyceride in the usual way, and the remainder of the procedure is identical with that described by Rode.

Hirt and Berchtold. Helv. Chim. Acta. 40 1928 (1957).

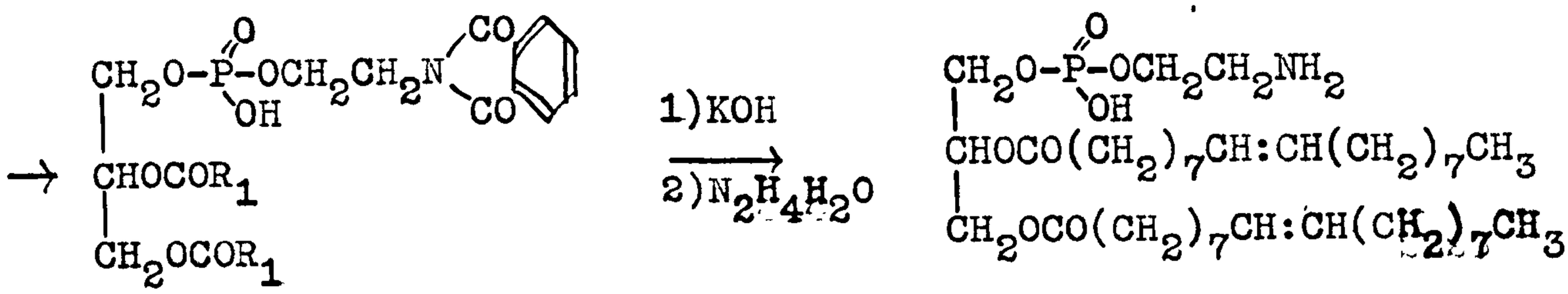


In the Report on the Symposium on "Phosphoric Esters and related compounds" held at the Chemical Society Anniversary Meeting at Cambridge, April 1957, Baer has described a method for the synthesis of L $\alpha$ dioleyl cephalin. The basic principle is similar to that described by Rose (loc.cit.), but it was necessary first of all to prepare an 1,2 unsaturated diglyceride, which was achieved very elegantly by acylating the 1 benzyl ether of glycerol with 9,10 dibromostearoyl chloride, removing the benzyl group by catalytic hydrogenolysis, and then debromimating with zinc dust in ether. The phosphorylation and subsequent stages were carried out in the same way as for the saturated compounds.





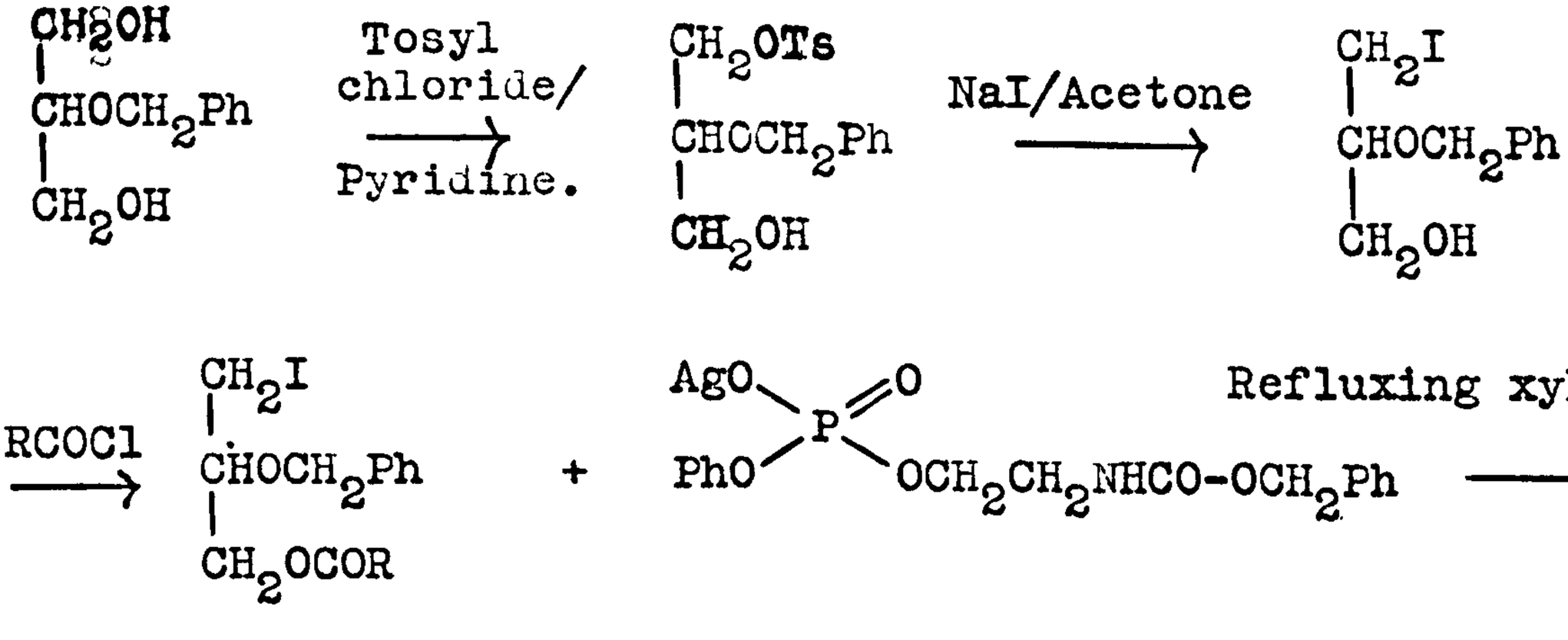

($R_1COO$-=Oleyl.)




1,2 Dioleyl cephalin.

Lysocephalins

The 1 phosphoryl 3 acyl and 1 phosphoryl 2 acyl lysocephalins have been prepared by Baylis, Bevan and Malkin (loc. cit.) by a modification of the method used for the diacyl cephalins; the reaction scheme is outlined below.

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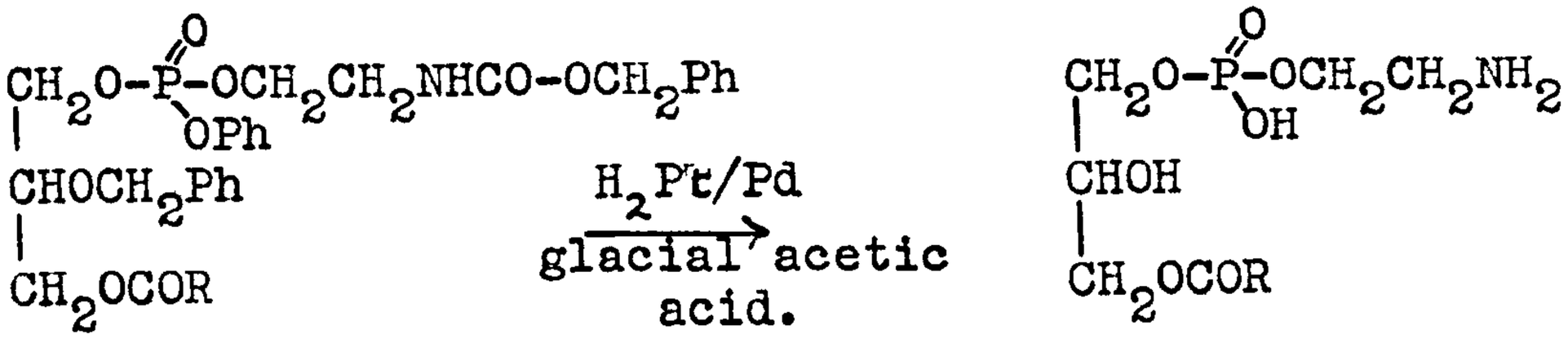


1-Phosphoryl 3-acyl lyso cephalin.

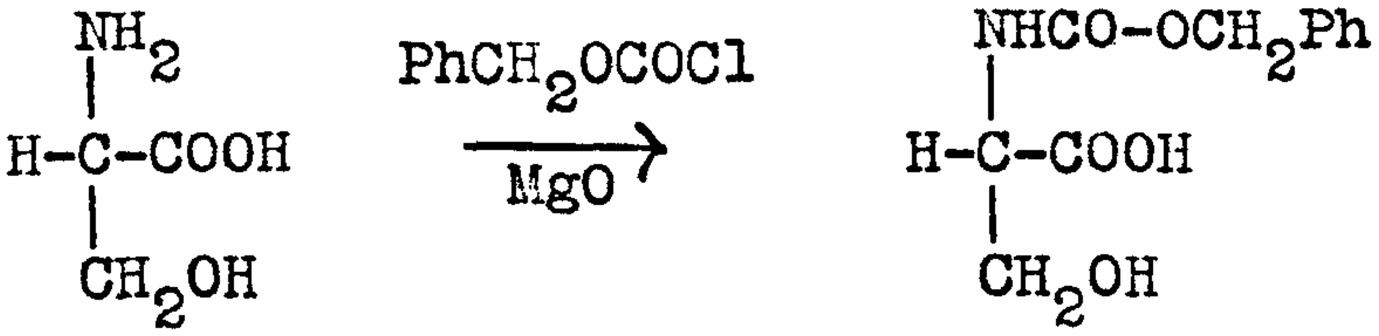
The 1 phosphoryl 2 acyl lysocephalins are prepared in a like manner using the 1 - benzyl ether of glycerol as the starting material.

Glycol cephalins.

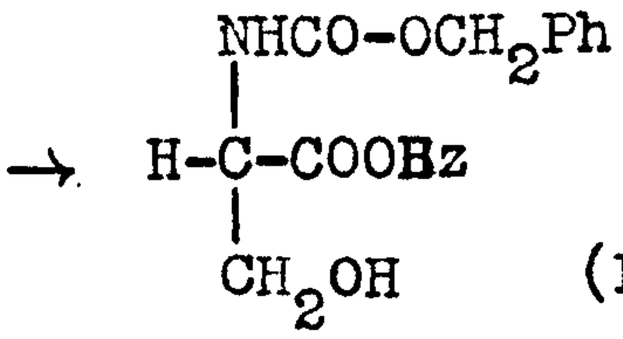
Baylis, Bevan and Malkin (loc.cit.) have also prepared the glycol cephalins by the silver salt method, using acyl glycoliodohydrin as the starting material (Bevan, Malkin and Smith J. 1043 (1955)).

Phosphatidyl Serine.

Phosphatidyl serine was first prepared by Baer and Maurukas (J.B.C. 212 25 (1955)). They synthesised the L 1,2 distearoyl phosphatidyl L serine, and found it to be identical in its properties with the natural product obtained from ox brain.

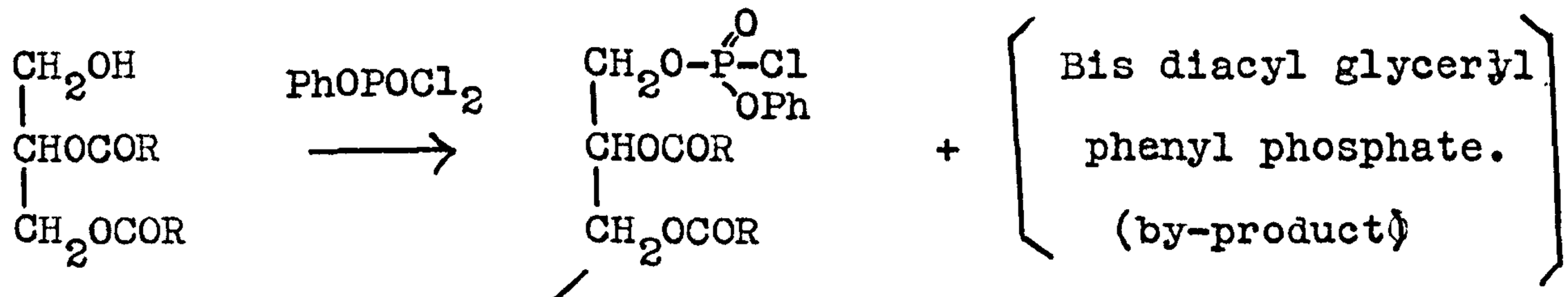



L serine




(Product "A")

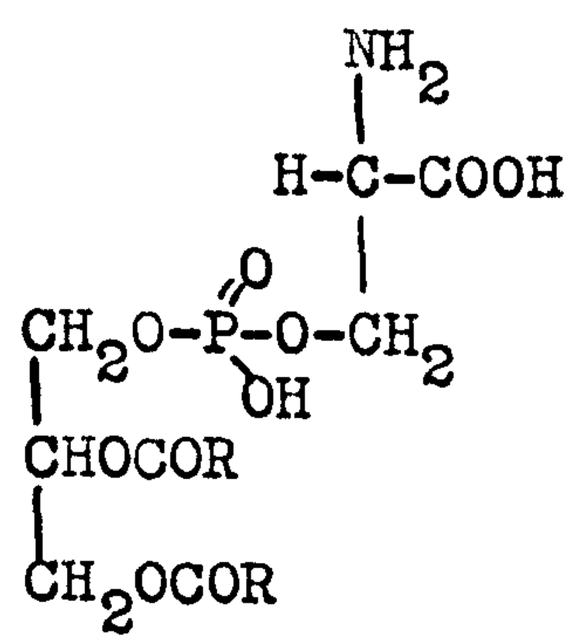
(N carbobenzoxy L serine benzyl ester)




D 1,2 distearin.



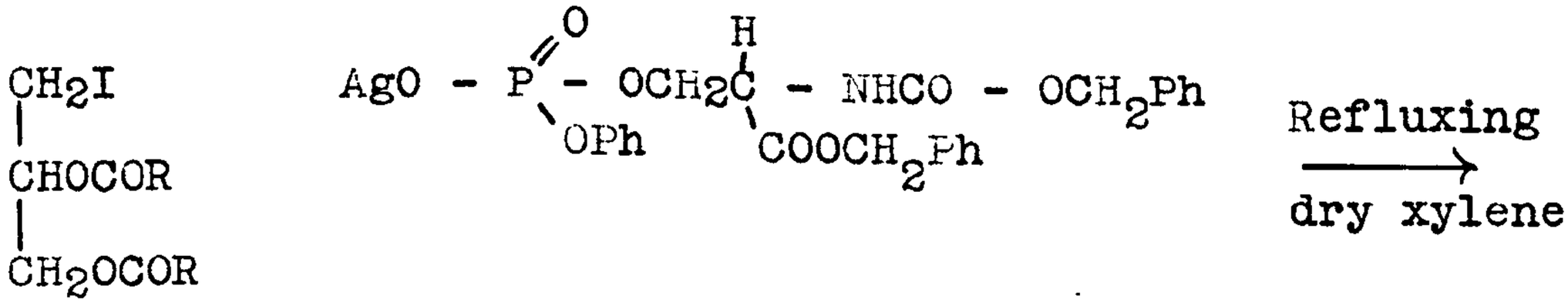





L 1,2 distearoyl phosphatidyl

L serine.

Recently, Bevan, Malkin and Tiplady (J. 3086 (1957)) have described a synthesis of the racemic form of phosphatidyl serine based on the reaction of DL 1,2 diacyl glycerol iodide with the silver salt of monophenyl phosphoryl N carbobenzoxy DL serine benzyl ester in refluxing dry xylene, followed by catalytic hydrogenolysis of the protecting groups.



# EXPERIMENTAL SECTION

PART I

## The Preparation of L 1,2 Diacyl cephalins and L 1,2 Diacyl Phosphatidyl L serine.

The Anomalous melting points of phosphatides.

Wide divergences in melting point have been recorded in the literature for various phosphatides. Until reliable synthetic methods had been devised this was perhaps understandable, since many of the "pure" compounds extracted from natural sources were mixtures incapable of separation by the techniques used. The solubilising effect of phosphatides and their tendency to form emulsions added to the difficulties.

The early syntheses published some thirty years ago did little to improve the situation. The lecithins and cephalins obtained were usually contaminated with the choline or ethanolamine salts of the corresponding phosphatidic acid, and in certain cases these salts were probably the major product.

Within the last ten years, however, unambiguous methods for the preparation of lecithin, cephalin, phosphatidyl serine and other phosphatides have been described. With reference to the cephalins, the following table summarises the melting point data obtained by various workers.

P.T.O.

| SYNTHETIC CEPHALIN. | | PREPARED BY:- |
|---|---|---|
| DL 1,2 DISTEAROYL 196° | 1,3 DISTEAROYL 198° | BEVAN AND MALKIN (1). |
| DL 1,2 DIPALMITOYL 198° | 1,3 DIPALMITOYL 206° | |
| DL 1,2 DIMYRISTOYL 207° | 1,3 DIMYRISTOYL 207° | |
| DL 1,2 DILAUROYL 210° | 1,3 DILAUROYL 208° | |
| L 1,2 DISTEAROYL 173-5° (With sintering | | BAER, MAURUKAS AND RUSSELL (2). |
| L 1,2 DIPALMITOYL 172.5-175° at lower | | |
| L 1,2 DIMYRISTOYL 175-177° temperatures) | | |
| DL 1,2 DIPALMITOYL 192-3°; 195-8° | | ROSE (3). |
| L 1,2 DISTEAROYL 180-182° | (Slight sintering at 130-135°) | BAER (4). |
| L 1,2 DIPALMITOYL 186-7° | | |
| L 1,2 DIMYRISTOYL 195-6° | | |

(1). Bevan and Malkin. J. 2667 (1951).

(2). Baer, Maurukas and Russell. JACS. 74 152 (1952).

(3). Rose. JACS. 69 1384 (1947)

(4). Baer. Canad. J. of Bioch. Physiol. 35 239 (1957).

A study of the table indicates that the L 1,2 Diacyl cephalins prepared by Baer et al. (2) melt at considerably lower temperatures(25°) than the DL 1,2 compounds prepared by other workers. Unless the lower melting point (and

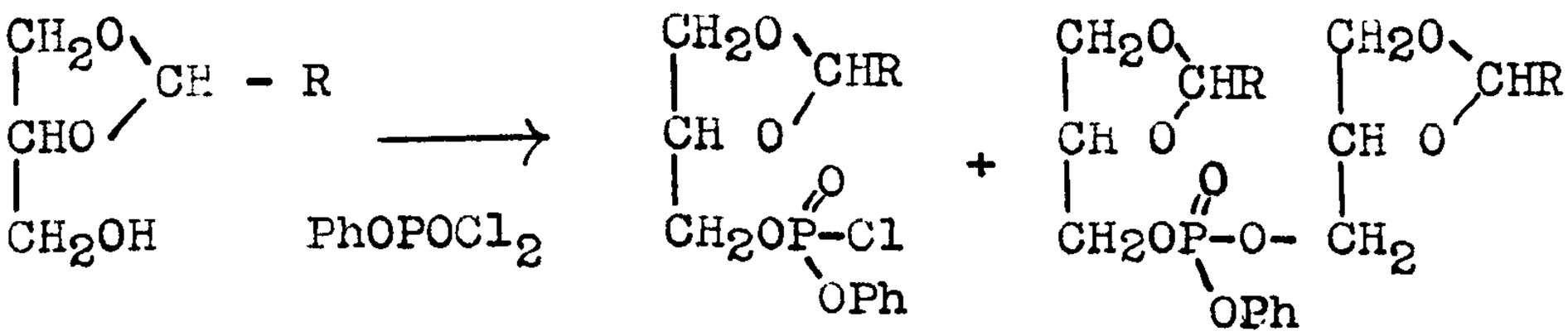
sintering below the melting point ) is an inherent property of the L forms, it would seem that the latter were less pure than those prepared by Bevan and Malkin (1) and by Rose (3). However, the analysis figures quoted by Baer et al.were very close to the theoretical values, indicating a high degree of purity. In view of this anomaly the synthesis of L 1,2 diacyl cephalins was undertaken by the author, using the silver salt method developed in these laboratories by Baylis, Bevan and Malkin (Report on Biochemical Problems of Lipids. Ghent. 1955. Butterworths ).

The L 1,2 diacyl cephalins thus obtained were identical with the racemic compounds in every respect, and no sintering at temperatures below the melting point was observed. The low melting points found by Baer et al. are therefore not due to the fact that they were dealing with optically active forms.

Since this work has been completed, Baer (4) has published details of the purification of the L 1,2 diacyl cephalins he obtained, by triturating with acetic acid and acetone, and recrystallising from chloroform - methanol. The substances had slightly raised melting points (see table on page 45 ). The new melting points, whilst still not altogether satisfactory, are now in closer agreement with those of other workers.

It seems likely that the method of preparing phosphatides using monophenyl phosphoryl dichloride as a phosphorylating

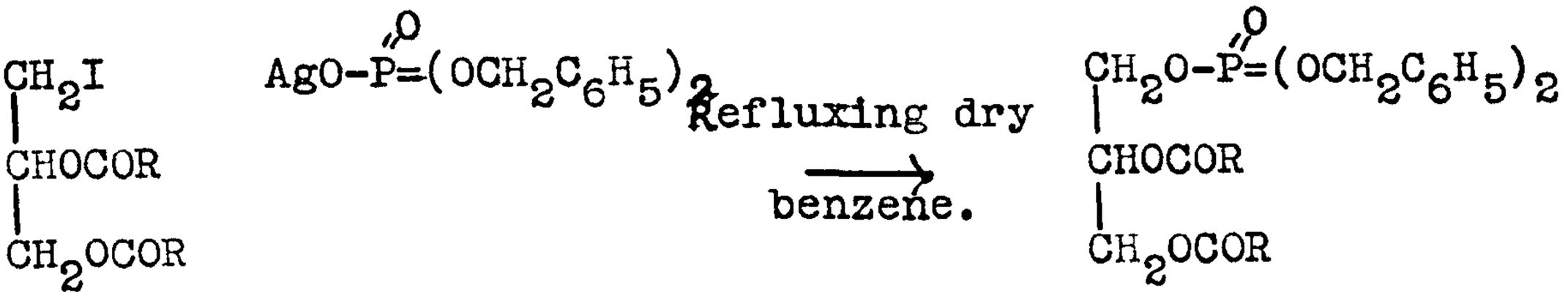
agent gives rise to less pure products than do other methods. Dr. Webley, of these laboratories, has prepared 1,2 acetal glyceryl phophatidyl ethanolamine by this method, and the product obtained sintered at 80°C, forming a meniscus at 225°C. Chromatographic fractionation on silicic acid eliminated the sintering, and a by product, bis 1,2 acetal glycerophosphoric acid was separated from the eluate. (D.J. Webley. Ph.D. Thesis. Bristol. 1957).




Reacts further with carbobenzoxy ethanolamine to give required product.

Bis compound by - product.

Although early syntheses employed the reaction of a silver salt with a halogen compound (cf. Kabashima), it was not until the work of Hessel, Morton, Todd and Verkade (Rec. trav. chim. 73 150 (1954)) that the yield and purity of the products obtained became satisfactory. These workers prepared 1,2 diacyl phosphatidic acids by reacting the well dried silver salt of Dibenzyl phosphate with 1,2 diacyl 3 iodo glycerol, in refluxing dry benzene in the dark.




1,2 Diacyl Phosphatidic Acid.

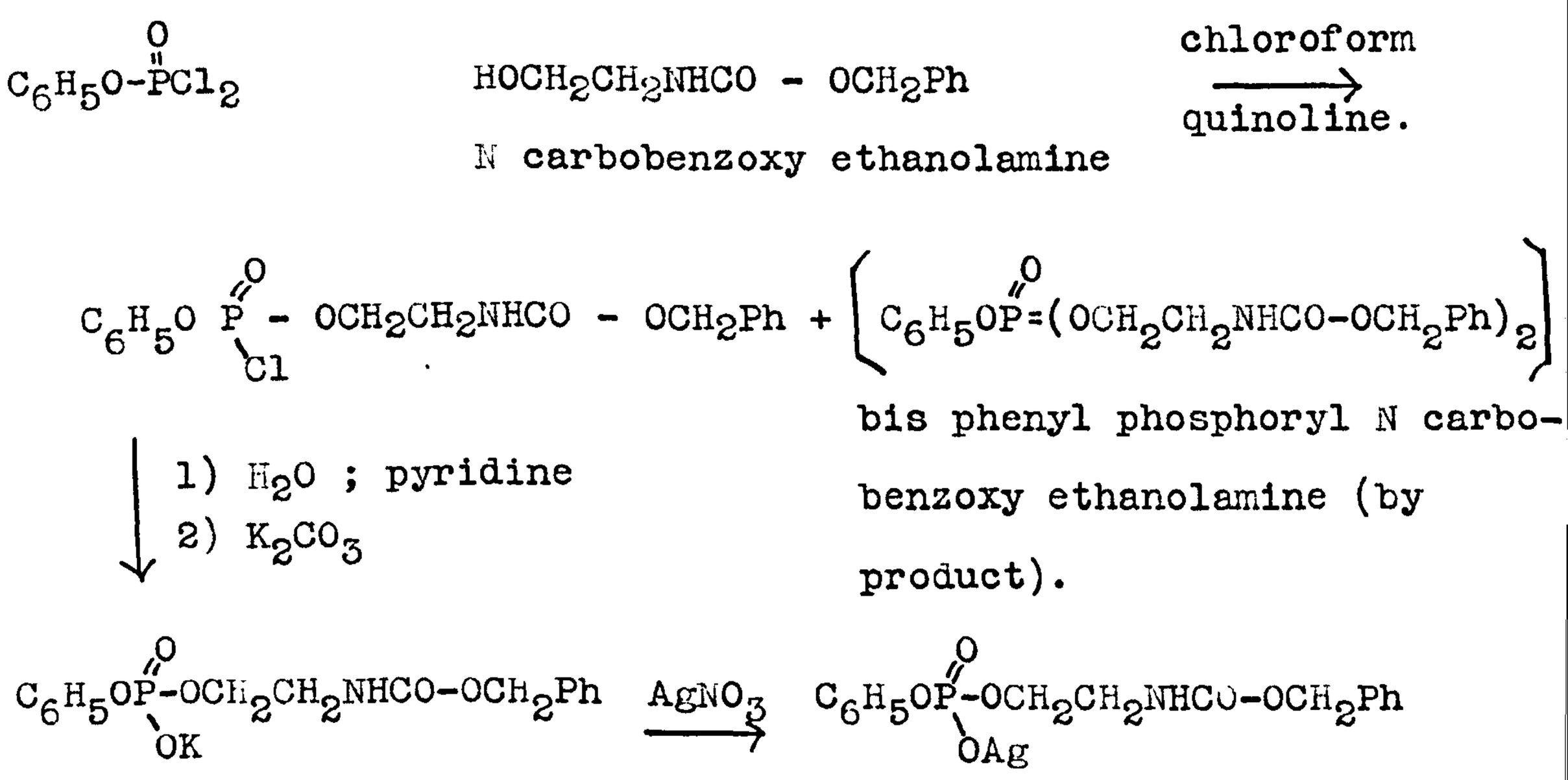
Using benzyl and phenyl silver salts, this method has now been extended in these laboratories to the synthesis of diacyl 1,2 glyceryl cephalins, glycol cephalins, cephalin analogues of straight chain alcohols, batyl and chimyl alcohols, $\alpha$ lyso cephalins, plasmalogens, phosphatidic acids and alkyl phosphates.

Discussion of Experimental Work.

Synthesis of L ∝ diacyl cephalins.

Preparation of the silver salt of monophenyl N carbobenzoxy ethanolamine phosphate.

This silver salt was used in all the syntheses of cephalins or cephalin analogues, and was prepared by the following route:-




The bis compound was extracted from the aqueous solution with ether after treatment with potassium carbonate. Owing to the formation of this bis compound the overall yield was of the order of 45%.

Preparation of L ∝ glycerol iodohydrin

Although 1,2 isopropylidene glycerol (acetone glycerol) can be prepared by the direct reaction of glycerol and acetone, the product is the racemic form. In order to obtain D acetone glycerol, and hence L ∝ glycerol iodohydrin, it was necessary to start with D mannitol, which was readily available as a commercial product.

From D mannitol 1,2,5,6 D diacetone mannitol was prepared by reaction with zinc chloride and acetone, and this product treated with lead tetra-acetate, which cleaved the adjacent free hydroxyl groups in the molecule producing D glyceraldehyde. This was then reduced to D acetone glycerol by one of two ways. viz;

a) Careful fractionation followed by reduction with lithium aluminium hydride. This method was quicker and required less preparation than the other, but the yield was inferior.

b) Catalytic hydrogenation in ethyl acetate using Raney nickel catalyst. When this method was used it was necessary to prepare the Raney nickel beforehand, and there were certain precautions which had to be taken to prevent the catalyst igniting.

The specific rotation of the liquid D acetone glycerol was measured using a Hilger and Watts polarimeter and 1 dm. tube. It was found to be + 14.2° at 20°C.

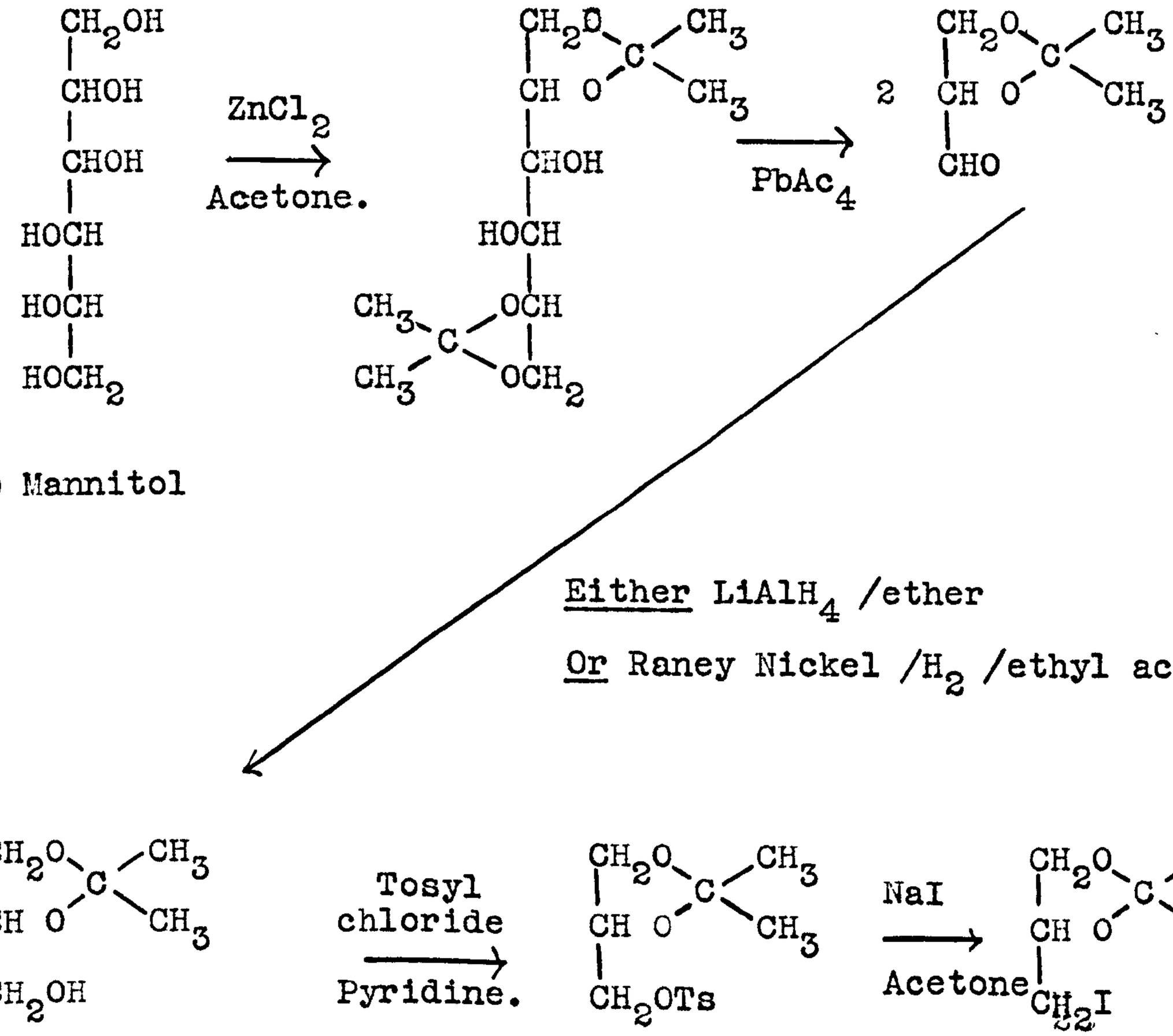
∝Tosyl D acetone glycerol was prepared from the product by reaction with Tosyl chloride and pyridine according to the method of Baer (JACS 609 (1948)). It was a viscous oil and was not purified, but reacted immediately with sodium iodide in refluxing acetone to yield ∝iodol acetoneL propylene glycol. A reflux time of 30 hrs. was allowed. The product was a liquid, b.pt. 68-9°C./6m.m. for which $[\alpha]_D^{20} = +53.0^\circ$ (in substance).

The final stage of the preparation, removal of the acetone group, was affected with aqueous ethanolic sulphuric acid for 20 hours, followed by neutralisation with barium carbonate and crystallisation of the L ∝ glycerol iodohydrin from chloroform/ 40-60 light petroleum. It was a white crystalline solid.m.p. 48-9°C $[\alpha]_D^{20} = -5.8^\circ$.

Overall yield from D mannitol 12%.

Yield from D acetone glycerol 41%.

(Reaction scheme overleaf).


CH2OH
CHOH
CHOH
HOCH
HOCH
HOCH2
ZnCl2
Acetone.
CH2O
C
CH3
CH O
CH3
CHOH
HOCH
CH3
C
OCH
CH3
OCH2
PbAc4
2
CH2O
C
CH3
CH O
CH3
CHO
Either LiAlH4 /ether
Or Raney Nickel /H2 /ethyl acetate.
D Mannitol
CH2O
C
CH3
CH O
CH3
CH2OH
Tosyl
chloride
Pyridine.
CH2O
C
CH3
CH O
CH3
CH2OTs
NaI
Acetone
CH2O
C
CH3
CH O
CH3
CH2I
D Acetone glycerol.
Dil. H2SO4
CH2OH
CHOH
CH2I
L∝ Iodohydrin.

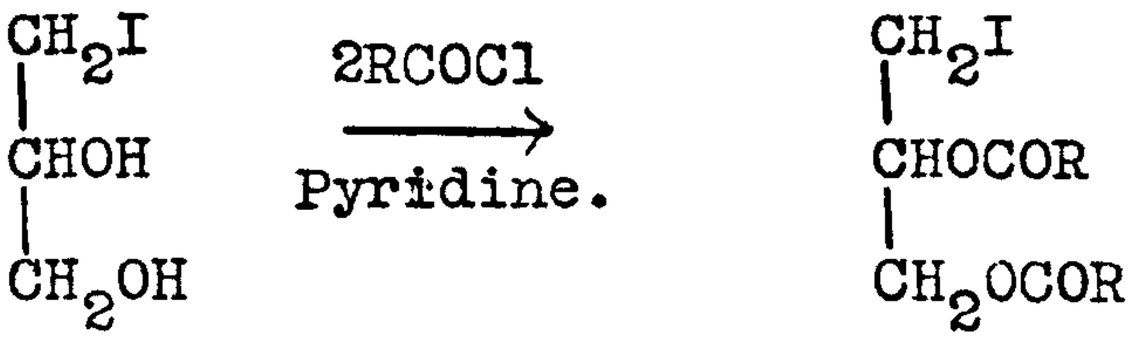
Preparation of L 1,2 diacyl glyceryl cephalins.

The method used for the L 1,2 diacyl glyceryl cephalins followed that employed by Baylis, Bevan and Malkin (loc.cit.) for the racemic compounds. L 1,2 diacyl glycerol iodohydrin was prepared by acylating L ∝ iodohydrin with 2 equivalents of acid chloride and pyridine, and this product was added to a stirred suspension of the well dried silver salt in refluxing anhydrous benzene in the dark. After refluxing for 1½ hours the precipitated silver salts were removed by filtration and the L 1,2 diacyl glyceryl N carbobenzoxy ethanolamine phenyl phosphate was immediately subjected to catalytic hydrogenolysis in glacial acetic acid using a platinum oxide (Adams) - palladium black mixed catalyst. After filtration of the catalyst the solvent was evaporated and the L 1,2 diacyl cephalin purified by triturating several times with boiling ether to remove soluble impurities and then recrystallising from ethanol.

Overall yield 55% based on L ∝ iodohydrin.

The melting points and specific rotations of these synthetic cephalins are recorded in the following table

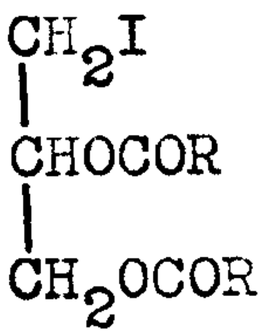
| Cephalin. | M.Pt. | $[\alpha]_D^{20}$ |
|---|---|---|
| L 1,2 distearoyl. | 196°C | + 5.4° |
| L 1,2 dipalmitoyl. | 198°C | + 5.0° |
| L 1,2 dimyristoyl. | 208°C | + 5.6° |

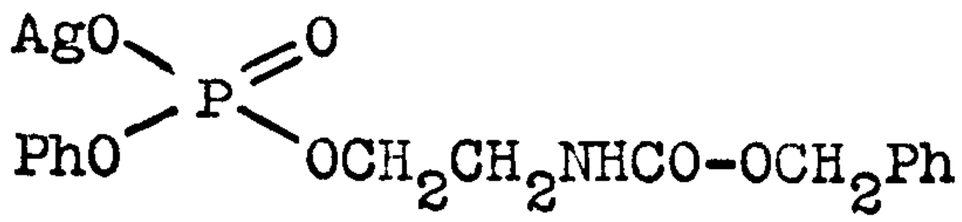


L $\alpha$ iodohydrin.

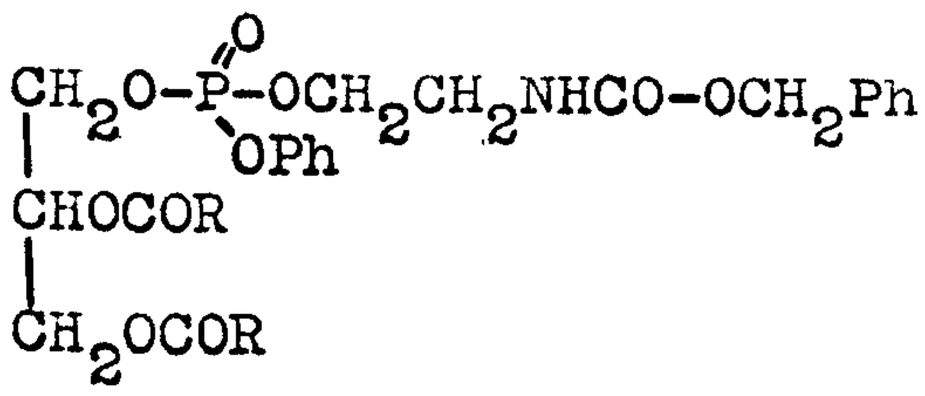


L 1,2 Diacyl iodohydrin.

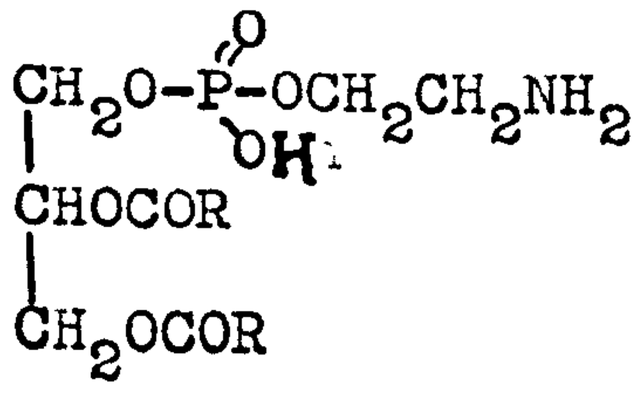




Refluxing benzene.



$H_2$ Pt/Pd



L 1,2 Diacyl cephalin.

Synthesis of L ∝ phosphatidyl serine.

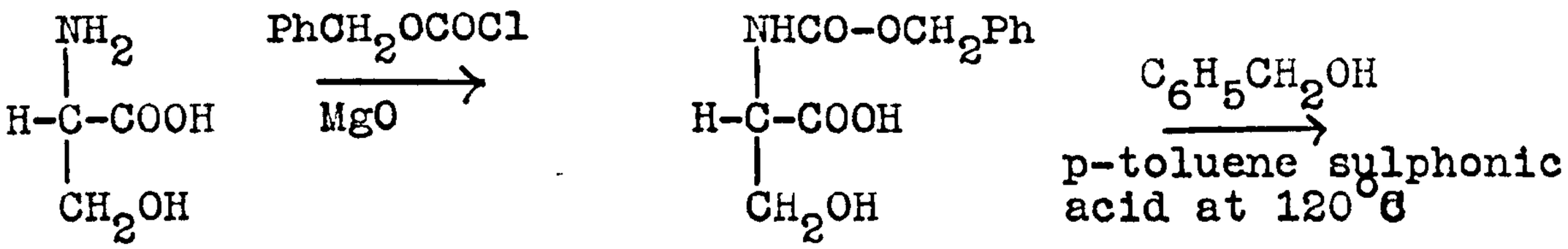
The method of synthesis of phosphatidyl serine by means of a silver salt reaction with diacylated glycerol iodohydrin was first developed in these laboratories by Bevan, Malkin and Tiplady (J. 3086 (1957)) for the DL form. The author has extended this method to the synthesis of L 1,2 diacyl glyceryl phosphatidyl L serine. The properties of the product correspond closely with those described by Baer and Maurukas (J.B.C. 212 25 (1955)).

Preparation of the silver salt of monophenyl N carbobenzoxy L serine benzyl ester phosphate.

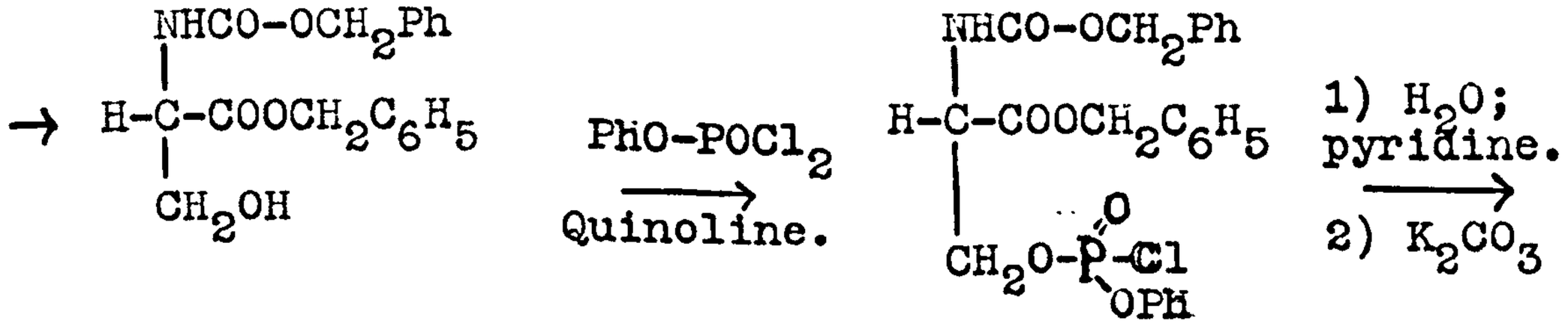
N - carbobenzoxy L serine was prepared according to the method of Baer and Maurukas (loc. cit.) using carbobenzoxy chloride, magnesium oxide and L serine. This was then esterified with benzyl alcohol at 120°C, using p - toluene sulphonic acid as a catalyst and removing the water produced at the pump. This method was superior to that used by Baer and Maurukas in that it was a single stage method, and avoided the use of benzyl bromide and sealed tubes. The yield was of the same order.

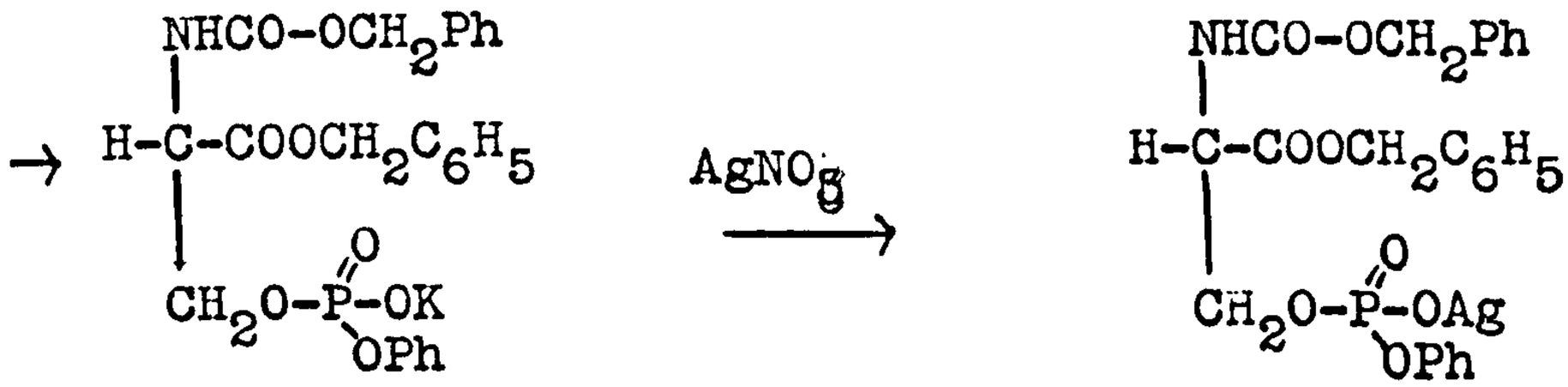
The N carbobenzoxy L serine benzyl ester was subsequently phosphorylated with monophenyl phosphoryl dichloride and quinoline, then hydrolysed to the free acid with water, and converted to the potassium salt with potassium carbonate. After

purification this was decomposed with silver nitrate solution yielding an oily precipitate of the silver salt of monophenyl N carbobenzoxy L serine benzyl ester phosphate. This was separated by decanting off the mother liquor and dried over phosphorus pentoxide in vacuo.




L serine.



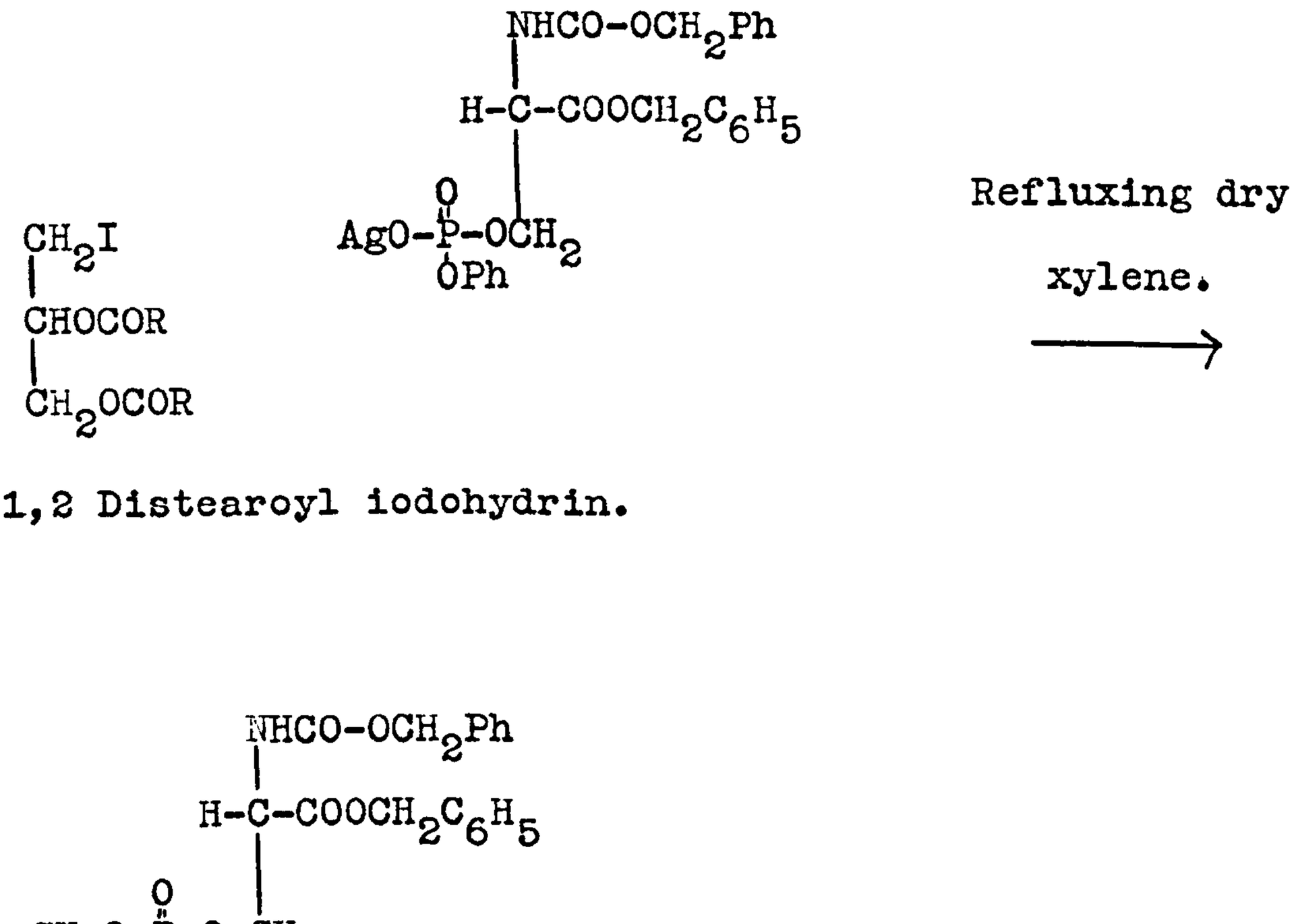
Preparation of 1,2 diacyl L glyceryl phosphatidyl L serine.

Diacyl L glyceryl iodohydrin (prepared as for the synthesis of the L 1,2 diacyl cephalins) was reacted in the dark with the well dried silver salt of monophenyl N carbobenzoxy L serine benzyl ester phosphate in refluxing dry xylene. After 15 minutes the solution was cooled and filtered, and after evaporation of the solvent the intermediate was hydrogenolysed in glacial acetic acid solution using a mixed Adams' platinum oxide - palladium black catalyst. The product, 1,2 diacyl L glyceryl phosphatidyl L serine was triturated with boiling ether and reprecipitated from its chloroform - glacial acetic acid solution with ether.

1, 2 Distearoyl L glyceryl phosphatidyl L serine was a fine white powder. m.p. 159 - 161°C with decomposition.

$[\alpha]_D^{19.5} = -19.7^\circ$. Yield from iodide 57%.

(Reaction scheme overleaf).


NHCO-OCH2Ph
H-C-COOCH2C6H5
O
AgO-P-OCH2
OPh
CH2I
CHOCOR
CH2OCOR
Refluxing dry xylene.
L 1,2 Distearoyl iodohydrin.
NHCO-OCH2Ph
H-C-COOCH2C6H5
O
CH2O-P-O-CH2
OPh
CHOCOR
CH2OCOR
H2Pt/Pd
glacial acetic acid.
NH2
H-C-COOH
O
CH2O-P-O-CH2
OH
CHOCOR
CH2OCOR
1,2 Distearoyl L glyceryl phosphatidyl L serine .

## Experimental.

### Solvents and Reagents.

If dry solvents were specified as being used, they were prepared as follows.

Chloroform. - Commercial chloroform was freed from alcohol by washing with water, then washed with concentrated sulphuric acid and then with water again. It was then shaken with solid calcium chloride until the opalescence had disappeared, and dried over phosphorus pentoxide. It was distilled shortly before use.

Benzene, Xylene and Ether. - these were dried by standing over sodium wire.

Pyridine and Quinoline. - Pyridine was dried by refluxing with solid sodium hydroxide for thirty minutes and then distilling from the sodium hydroxide. Quinoline was purified by distillation alone, overheating being avoided. Dry pyridine and quinoline were stored in a dessicator over concentrated sulphuric acid.

Acetone. - this was allowed to stand over anhydrous calcium sulphate.

Alcohols. Anhydrous ethyl alcohol was prepared by refluxing magnesium with a small quantity of alcohol thus forming magnesium ethylate, followed by the addition of a larger quantity of commercial "absolute" alcohol. The moisture present reacts

with the magnesium ethylate, forming ethyl alcohol and insoluble magnesium hydroxide.

$$Mg + 2C_2H_5OH = Mg(OC_2H_5)_2 + H_2 \quad -(1)$$

$$Mg(OC_2H_5)_2 + 2H_2O = Mg(OH)_2 + 2C_2H_5OH - (2)$$

To start reaction (1) it was necessary to add a little solid iodine to the magnesium.

After drying the ethyl alcohol was distilled off using carefully dried apparatus.

Methyl alcohol may be dried by a similar procedure.

The long chain fatty acids used were Kahlbaum's "K" or highly purified acids supplied by Price's of Bromborough Ltd. These were further purified where necessary by high vacuum fractionation of the ethyl esters.

Note on measurement of specific rotation.

The optical rotation of all the optically active substances prepared was measured using a Hilger and Watts polarimeter and a 1 dm.(10cm.) tube. Where the substances were liquids the rotations are quoted "in substance", that is, for a 1 dm. length of the liquid. In such cases the specific rotation $[\alpha]_D$ (for the sodium D lines used throughout as a monochromatic light source) is given by the following formula:-

$$\text{Specific Rotation} = \frac{\text{Actual Rotation}}{\text{Density of liquid}}$$

It is necessary to quote the temperature at which the measurement was made since the densities of liquids, and their rotations, change with temperature. The temperature is usually added as a suffix e.g. at 20°C $[\alpha]_D^{20}$

In the case of solids it is necessary to measure the rotation of a solution of the substance. The solvent must be specified, and the concentration of the solid in the solvent must also be determined. The specific rotation is given by the following expression:-

$$[\alpha]_D^{20} = \frac{100.\ (\text{Actual Rotation})}{lpd}.$$

where l = length of tube in dm. (1 usually).

p = no. of grams: of solid in 100 ml. of solvent.

d = density of solution.

In order to determine the density of the solution it was necessary to weigh a known volume. This can be done quite accurately by weighing a standard graduated flask empty and filled to the mark with solution.

Owing to the relatively low solubilities of certain compounds, such as cephalins and phosphatidyl serine, fairly low concentrations of these substances had to be used when making measurements. This resulted in the actual measured rotation of the solution being very small indeed - of the order

of 0.1 - 0.2°. An appreciable error could thus have been introduced into the result, and this was minimised by taking the average of six readings for the actual rotation.

## Preparation of L ∝ cephalins.

### Preparation of 1,2,5,6 D acetone mannitol.

(According to the method of Biochemical Preparations Vol.II).

105 gm. ( 0.77 mole) of zinc chloride stick was added to 520ml. of dry acetone and the mixture was swirled until all the zinc chloride had dissolved. The flask was set aside to allow the solution to cool to room temperature and the sediment to settle.

65 gm. (0.36 mole) D mannitol were placed in a two litre flask equipped with a mechanical stirrer. The stirrer was set in motion and the zinc chloride/acetone solution was added by decantation, keeping back the insoluble material. The stirring was continued two hours until most of the D mannitol had dissolved. At the end of this time the unreacted D mannitol was filtered off.

The filtrate was now poured as rapidly as possible into a vigorously stirred mixture of 130 gm. anhydrous potassium carbonate in 130 ml. water and 520 ml. of ethanol free ether, a three litre flask being used. The mixture was stirred for a further 40 minutes, and the precipitate of zinc carbonate washed with 200 ml. of an equal mixture of ether/acetone. The combined solution was concentrated under reduced pressure and dried in vacuo at 60 - 70°C for 1 hour.

The resulting solid was extracted with hot benzene, and the

insoluble monoacetone compound was removed by filtration. After cooling the benzene solution the 1,2,5,6 D acetone mannitol was precipitated by the addition 60/80 light petroleum.

Yield 42 gm. (45%). m.p. 119 - 120°C.

Preparation of lead tetraacetate.

(The method used is described in "A Text Book of Practical Organic Chemistry" by A.I. Vogel).

200 gm. Red lead (previously dried in vacuo over concentrated sulphuric acid) were added portionwise to a stirred mixture of 370 gm. glacial acetic acid and 125 gm. acetic anhydride, the temperature being maintained between 55 - 60°C and not exceeding 65°C. After all the red lead had been added the solution was allowed to cool, and the crystalline deposit of lead tetra-acetate was filtered and recrystallised from glacial acetic acid containing a little acetic anhydride. The product was dried in a vacuum dessicator over potassium hydroxide pellets.

The yield was 100 gm.

Preparation of D acetone glyceraldehyde.

42 gm. ( 0.16 mole ) 1,2,5,6 D Acetone mannitol were dissolved as far as possible in 500 ml. dry benzene, and 71 gm. (0.16 mole ). lead tetra-acetate were added with stirring in one portion. Lead diacetate separated as a white precipitate. The excess of lead tetra-acetate was just neutralised by adding small

portions of D acetone mannitol and testing with starch-iodide paper.

After 1 hour the precipitated lead salts were filtered off, and the solution freed from benzene by evaporating it down under an efficient fractionating column at approximately 10 cm. of mercury pressure and at a temperature not exceeding 45°C. Care was necessary as the boiling point of D Acetone glyceraldehyde was 35 - 42°/8 - 11 m.m. of mercury.

Reduction with Lithium Aluminium Hydride.

12 gm. of Lithium Aluminium Hydride were suspended in 250 ml. dry ether in a flask equipped with magnetic stirrer and reflux condenser, all exits being guarded with calcium chloride tubes, and the D acetone glyceraldehyde was added dropwise at such a rate as to cause the ether to reflux gently. After this had all been added the solution was refluxed 30 minutes and then left overnight.

50 ml. ethyl acetate were now added to decompose the excess lithium aluminium hydride, and then moist ether and finally water were cautiously added to break down the lithium-aluminium complex, the colour changing from grey to white. The precipitated mixture of lithium and aluminium hydroxides was removed by filtration, washed well with acetone, and the filtrate and washings were dried with anhydrous sodium sulphate.

After removal of the solvents under reduced pressure the D acetone glycerol was distilled at the pump. b.pt. 79°C/15m.m. Yield 30.5 gm. (75%).

$[\alpha]_D^{20} = +14.2^\circ$ in substance.

Preparation of ∝ tosyl D acetone glycerol.

22.8 gm ( 0.17 mole ) of D acetone glycerol were added with shaking to a cooled mixture of 32.8 gm. ( 0.17 mole) p toluene sulphonyl chloride and 18 ml. dry pyridine. The mixture was kept at 0° C for 60 minutes, and room temperature for 48 hours. The whole was then poured into 500 ml. ice water, the supernatant liquid immediately decanted and the heavy oil dissolved in ether, and washed with sodium carbonate solution. The ethereal solution was then dried with anhydrous sodium sulphate.

After removal of the drying agent and distilling off the ether in vacuo at a temperature not exceeding 35°C, the resulting viscous oil was stripped on the"hivac" pump for four hours (below 35° C) to remove excess pyridine.

The crude ∝ tosyl D acetone glycerol (43.0 gm. 87% ) was not further purified but used immediately for the preparation of ∝ iodo acetone L propylene glycol.

Preparation of ∝ iodo acetone L propylene glycol.

43.0 gm. ( 0.15 mole ) of ∝ tosyl D acetone glycerol and 67.5 gm. ( 0.45 mole; 3 equivalents) of dry sodium iodide were refluxed together in 300 ml. dry acetone for 24 hours with stirring. The precipitate of sodium p - toluene sulphonate was filtered off, a further 7 gm. sodium iodide added, and refluxing continued for 5 hours. After re-filtering the solution was concentrated to dryness at a temperature not exceeding 40°C.

The dark reddish brown solid residue was extracted 7 - 8 times with 100 ml. portions of warm ether, and the combined ethereal extracts were freed from iodine by washing with sodium thiosulphate solution. The ethereal solution was dried with sodium sulphate and concentrated under reduced pressure to yield a clear yellow oil.

This was distilled under high vacuum. b.p. 48 - 9°C/1.m.m. Yield 28.5 gm. (79%). $[\alpha]_D^{20} = + 52.5^\circ$ in substance.

Hydrolysis of above compound to ∝ iodohydrin L propylene glycol.

28.5 gm. ( 0.12 mole ) ∝ iodo acetone L propylene glycol were dissolved in 75 mls. 85% ethanol, and 2.5 mls. 5N sulphuric acid were added. The solution was allowed to stand at room temperature overnight, and was then diluted with 10 mls. of water and refluxed 5 minutes to ensure that complete hydrolysis

had occurred.

After cooling, the solution was neutralised by stirring with solid barium carbonate, filtered carefully through a "Filtercel" pad to remove insoluble barium salts, and concentrated to dryness in vacuo at a temperature between 35 - 45°C. The residue was dissolved in ether (in which it was completely soluble), dried over sodium sulphate, and the ether was evaporated. The residual solid was recrystallised from chloroform/40 - 60 light petroleum, yielding 17.4 gm. (73%) of pure ∝ iodohydrin L propylene glycol.m.p. 48 - 9°C.

$[\alpha]_D^{20}$ = - 5.8° in dry ethanol.

Preparation of the silver salt of monophenyl N carbobenzoxy, ethanolamine phosphate.

N-Carbobenzoxy ethanolamine ( 50.4 gm ; 0.26 mole) in 300 ml. dry chloroform was added dropwise over 8 hours to a stirred ice cold mixture of monophenyl phosphoryl dichloride (54.7 gm ;0.26 mole), quinoline (34.4 gm.) and dry chloroform (50 ml.). All exits in the apparatus were guarded by calcium chloride tubes. When the addition was complete the mixture was stirred at room temperature for 12 hours, and the chloroform was then distilled off under reduced pressure at a temperature below 40°C.

20.5 gm. pyridine and 10 ml. water were now added to the cooled mixture ( the latter dropwise with stirring), and

stirring was continued for a further 24 hours, when the solution was neutralised by the addition of 150 ml. of water and 57.3 gm. (3 equivalents) of anhydrous potassium carbonate. The neutralised aqueous solution was extracted with ether (3x200 ml.) to remove bis N-carbobenzoxy ethanolamine phenyl phospate, and evaporated to dryness at a temperature below 40°C.

The residue was extracted with 200 mls. ethanol, filtered free of potassium chloride and evaporated to dryness below 40°C to yield a viscous oil, weight 56.3 gm. This was the required potassium salt.

The oil was dissolved in 50 ml. water, and made just acid to litmus with 2N nitric acid. A solution of 28.0 gm. (5% excess) of silver nitrate in 30 ml. water was added with stirring in the dark, and the precipitate was allowed to settle for 12 hours. After this it was filtered, washed consecutively with water and with ethanol, and dried to constant weight over phosphorus pentoxide in vacuo.

Yield 52.0 gm. (44%) m.p. 155°C.

Analysis. Found Ag 23.6%.

Calculated for $C_{16}H_{17}O_6NPAg$ Ag 24.25%.

The silver salt was kept in the dark at all stages of its preparation.

Preparation of long chain acid chlorides.

These were prepared by heating the pure fatty acid with oxalyl chloride or thionyl chloride, the latter preferably being purified first by fractionation from dry quinoline followed by distillation from pure linseed oil.

Preparation of myristoyl chloride.

(The details of this preparation are typical for any long chain acid chloride).

8 gm. (0.035 mole) of pure myristic acid were placed in a round bottomed flask equipped with a reflux condenser and calcium chloride tube, and the flask and its contents were heated on an oil bath until the acid was molten. The theoretical weight of thionyl chloride (4.2 gm. 0.035 mole) was added, and the mixture heated in a fume cupboard at 100 - 110°C for 1 hour. A vigorous evolution of hydrogen chloride occurred. A further 4.2 gm. (0.035 mole ) thionyl chloride was added, and heating was continued for a further hour.

The excess thionyl chloride was then removed at the pump, and the crude acid chloride distilled in vacuo. b.pt. 120-130°C at 1 m.m. of mercury. Yield 8.0 gm. (93%).

Stearoyl chloride b.p. 155 - 160°/1 m.m. and Palmitoyl chloride b.p. 135 - 140°/1 m.m. were also prepared in this way.

Preparation of L 1,2 distearoyl 3 iodo glycerol.

To 1.75 gm. (0.00875 mole) of L $\alpha$ glycerol iodohydrin and 5 ml. dry pyridine in 15 ml. dry chloroform, 5.2 gm. (0.0173 mole) stearoyl chloride in 15 ml. dry chloroform were added with cooling and shaking. After standing overnight the chloroform was evaporated under reduced pressure and the residual oil dissolved in ether, washed successively with 5N sulphuric acid, saturated sodium bicarbonate solution and water (twice). The ethereal solution was then dried over sodium sulphate.

When the ether was distilled off 6.8 gm. solid residue remained, which on recrystallising twice from methanol yielded 5.6 gm. (90%) of pure L 1,2 distearoyl 3 iodo glycerol.

m.pt. 53 - 4°C.

Analysis. Found. C 63.5 H 10.3

$C_{39}H_{75}O_4I$ requires C 63.8 H 10.2

$[\alpha]_D^{20} = +2.7^\circ$ in chloroform/ethanol:5/4

In a similar manner the following were also prepared.

1,2 dipalmitoyl 3 iodo glycerol. m.pt. 45 - 6°C.

Analysis. Found. C 60.9 H 9.9

$C_{35}H_{67}O_4I$ requires C 61.0 H 9.9

$[\alpha]_D^{20} = +2.8^\circ$ in chloroform/ethanol: 2/1.

1,2 dimyristoyl 3 iodo glycerol. m.pt. 35 - 6°C.

Analysis. Found. C 60.1 H 9.6

$C_{31}H_{59}O_4I$ requires C 59.8 H 9.5

$[\alpha]_D^{20} = +2.7^\circ$ in chloroform/ethanol: 2/1.

Reaction of L 1,2 distearoyl 3 iodo glycerol ( $\alpha,\beta$ distearoyl L$\alpha$ iodohydrin) with the silver salt of monophenyl N carbobenzoxy ethanolamine phosphate.

4.5 gm. (0.00613 mole) of 1,2 distearoyl L $\alpha$ iodohydrin dissolved in the minimum amount of dry benzene was added to a vigorously stirred suspension of 3.1 gm. (0.0067 mole; 10% excess) of the silver salt of monophenyl N carbobenzoxy ethanolamine phosphate in 100 ml. refluxing dry benzene in the dark. The mixture was refluxed and stirred for 1½ hours, allowed to cool, and filtered through a "Filtercel" pad to remove precipitated silver salts. After washing the precipitate with a little dry benzene the combined filtrates were evaporated to dryness below 40°C.

The residue was dissolved in ether, washed with saturated sodium bicarbonate solution to remove acidic by-products, then twice with water, and the ethereal solution was dried over sodium sulphate.

After filtration of the dessicant and removal of the solvent under reduced pressure, an oily residue was obtained which

solidified when cooled. This was used without further purification for the next stage of the synthesis.

Hydrogenolysis of protecting groups.

The residue was dissolved in 160 ml. glacial acetic acid and shaken in an atmosphere of hydrogen in the presence of 1.5 gm palladium black/1.5 gm. Adams's platinum oxide catalyst. The hydrogen pressure was slightly above atmospheric. When uptake of hydrogen ceased (1,200 ml. taken up ; approximately double the theoretical amount), the flask was evacuated, and the catalyst was filtered and washed well with chloroform to dissolve precipitated cephalin. The combined filtrates were evaporated to dryness (bath temperature $< 40^{\circ}C$ ), benzene being added to remove the last traces of acetic acid azeotropically. The solid residue was extracted twice with 30 ml. portions of boiling ether, and then recrystallised from ethanol.

L 1,2 distearoyl cephalin. 2.7 gm. (60%) m.p. $196^{\circ}C$.

| | | | | | |
|---|---|---|---|---|---|
| Analysis. | Found | C 65.6 | H 10.8 | N 2.0 | P 3.9 |
| $C_{41}H_{82}O_8NP$ | requires | C 65.9 | H 11.0 | N 1.9 | P 4.1 |

$[\alpha]_D^{20} = +5.4^{\circ}$ in chloroform/glacial acetic acid 4/1.

The following were also prepared as above:

L 1,2 dipalmitoyl cephalin. m.p. 198°C.

Analysis. Found C 64.0 H 10.8 N 2.2 P 4.8

$C_{37}H_{74}O_8NP$ requires. C 64.2 H 10.7 N 2.0 P 4.5

$[\alpha]_D^{20}$ = + 5.0° in chloroform/glacial acetic acid 4/1.

L 1,2 dimyristoyl cephalin m.p. 208°C.

Analysis. Found C 62.2 H 10.6 N 2.2 P 5.2

$C_{33}H_{66}O_8NP$ requires. C 62.4 H 10.4 N 2.2 P 4.9

$[\alpha]_D^{20}$ = + 5.6° in chloroform/glacial acetic acid 9/1.

X-Ray investigation of the L 1,2 Diacyl cephalins.

The X-ray spacings of the L 1,2 diacyl cephalins were photographed using a circular camera. (see Diagram Plate I ). The source of X-radiation was a Philips tube with a water cooled anti-cathode, which produced X-rays of 1.54 Å wavelength. Other radiation was filtered out with a nickel filter.

The crystalline specimen was mounted in a 1 m.m. diameter cellulose acetate tube and rotated in the X-ray beam by an electric motor. When the X-ray film was developed, characteristic circular spacings were produced (Plate I ), consisting of two dense circles (Side spacings) and a larger number of faint circles (Long spacings).

For a homologous series of long chain compounds, the side

spacings of all members of the series are similar. The long spacings, however, vary with the length of the chain. The side spacings alone were measured in this instance.

The diameter of the spacing circles (1 cm) was found using an instrument carrying a pointed needle, which was moved over the film surface by a screw thread. The needle holder moved along a scale graduated in millimetres, and a drum graduated to 0.01 m.m. was attached to the screw handle.

Calculation of results.

Example ; L 1,2 distearoyl cephalin.

| | | | |
|---|---|---|---|
| Readings L.H. | 16.1329 | Readings R.H. | 18.1406 |
| of centre. | 16.1160 | of centre. | 18.1731 |
| Mean | 16.1245 | Mean | 18.1569 |

Difference of means (1) = 2.0324 cm.

From the diagram (Plate I )

$\theta$ = Glancing angle ; l = diameter of spacing circles.

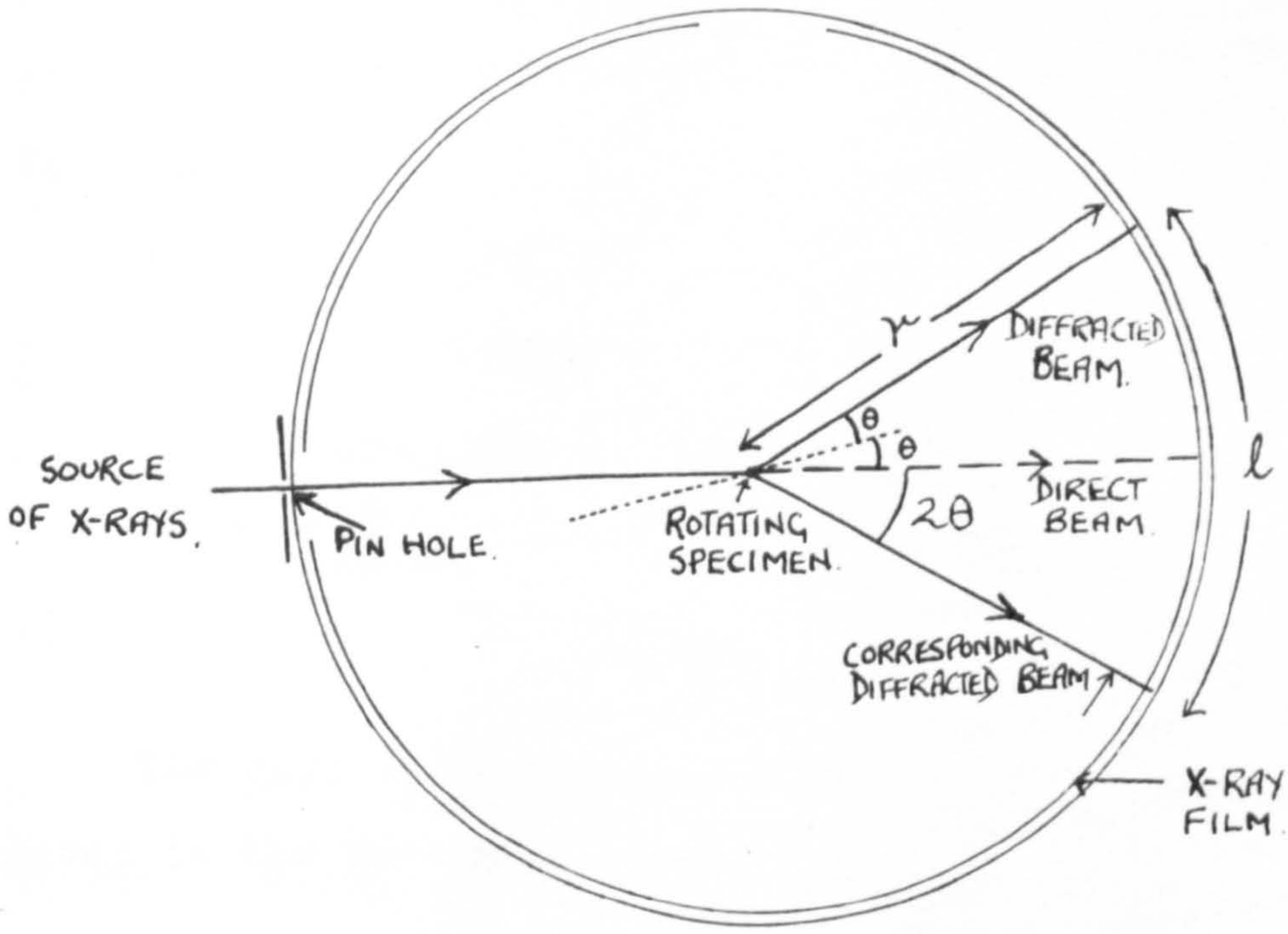
D = 2r = Diameter of camera (57 cm.).

$$4\theta_{\text{radians}} = \frac{l}{r} \qquad \therefore \theta_{\text{radians}} = \frac{l}{4r} = \frac{l}{2D}$$

$$\therefore \theta_{\text{degrees}} = \theta_{\text{radians}} \frac{180}{\pi} = \frac{l}{2D}\left(\frac{180}{\pi}\right)$$

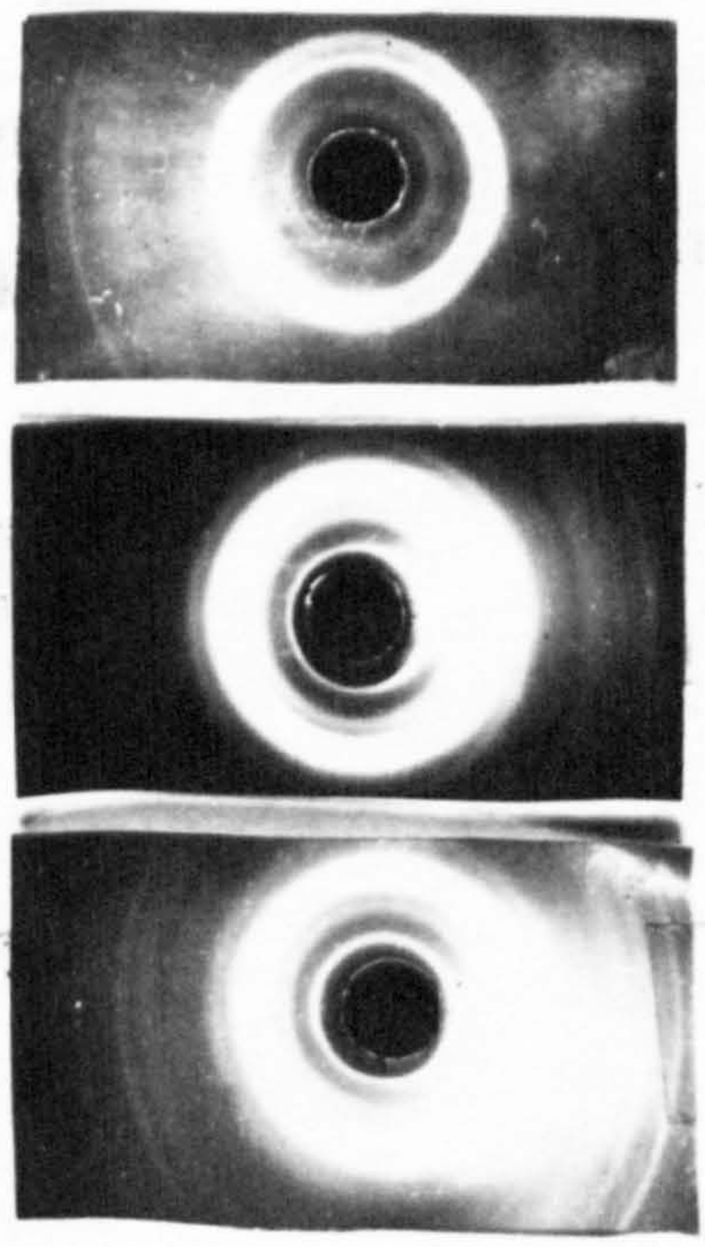
$$= l.\left(\frac{90}{\pi D}\right) \qquad \left(\frac{90}{\pi D} = \text{Constant for camera}\right)$$

PLATE I




θ = GLANCING ANGLE FOR X-RAYS.

DIAGRAM OF X-RAY APPARATUS.



L 1,2 DIMYRISTOYL CEPHALIN.

L 1,2 DIPALMITOYL CEPHALIN.

L 1,2 DISTEAROYL CEPHALIN.

For 1,2 Distearoyl cephalin. $\theta = 2.0324 \quad \frac{90}{\pi.57} = \underline{10^{\circ}\ 13'}$

By Bragg's Law $n\lambda = 2d \sin\theta$

Where n = Order of reflection (1 for side spacings)

$\lambda$ = Wavelength of radiation (1.54 Å ).

d = Magnitude of spacing in Å.

$$d = \frac{n\lambda}{2 \sin\theta} = \frac{1.54}{2 \times 0.1774}$$

$$= \underline{4.3}\ \text{Å}$$

The side spacings for the three L 1,2 diacyl cephalins are given in the following table.

| Cephalin. | Spacing Å | |
|---|---|---|
| L 1,2 Distearoyl. | 3.86 | Strong. |
| | 4.32 | Strong. |
| L 1,2 Dipalmitoyl. | 3.90 | Strong. |
| | 4.36 | Strong. |
| L 1,2 Dimyristoyl. | 3.84 | Strong. |
| | 4.52 | Strong. |

Attempts to photograph the long spacings for these compounds (using the Muller spectrograph) were uniformly unsuccessful, possibly because they were not sufficiently crystalline. For the side spacings glacial acetic acid was used as the crystallising solvent. Tetrahydrofuran (used by Bevan and Malkin J. 2667 (1951)) and dioxan (used by Baer,

Maurukas and Russell (JACS 74 152 (1952)) gave amorphous specimens only.

Preparation of L 1,2 distearoyl glyceryl phosphatidyl L serine.

Preparation of N carbobenzoxy L serine.

The following method is due to Baer and Maurukas J.B.C. 212 25 (1955).

5.5 gms. (0.032 mole) of freshly distilled carbobenzoxy chloride were added dropwise over 20 minutes to a vigorously stirred ice cold mixture of 2 gm. (0.019 mole ) L serine, 2.6 gm. (0.065 mole) "Analar" magnesium oxide, 35 mls. water and 16 mls. ether. After the addition was complete the mixture was stirred at 0°C for 2 hours, and at room temperature for 30 minutes.

The resulting emulsion was filtered slowly through a pad of "Filtercel", and the ethereal and aqueous layers were separated, the aqueous layer being washed with 20 mls. ether to remove traces of carbobenzoxy chloride. The aqueous solution was then acidified with 5N hydrochloric acid to Congo Red, and cooled in the refrigerator overnight. The precipitated N carbobenzoxy L serine was filtered off, washed with a little cold water, and dried in vacuo. Extraction of the aqueous filtrate with ether, followed by drying of the ethereal solution with anhydrous sodium sulphate yielded a further small quantity of material.

Total yield of crude N carbobenzoxy L serine 3.6 gm. m.pt. 113 - 115°C.

This was recrystallised from 1;3 ethyl acetate - 40/60 light petroleum.

Yield of pure N carbobenzoxy L serine 3.4 gm. (75%). m.pt. 115 - 6°C. $[\alpha]_D^{20}$ = + 5.6° in A.R. glacial acetic acid.

Preparation of N carbobenzoxy L serine benzyl ester.

(Method of Bevan, Malkin and Tiplady J 3086 (1957)).

3.4 gm. (0.014 mole) N carbobenzoxy L serine, 18 ml. freshly distilled benzyl alcohol, and 0.2 gm. p toluene sulphonic acid were placed in a 50 ml. flask equipped for downward distillation, and the benzyl alcohol was distilled off at the pump from an oil bath at a temperature not exceeding 130°C. The process was then repeated using a similar volume of fresh benzyl alcohol. Final traces of benzyl alcohol were removed by vacuum stripping at 100 - 120°C for 4 hours.

The residue was dissolved in 50 ml. of ether, washed successively with one 25 ml. portion of saturated sodium bicarbonate solution, and two 50 ml. portions of water, and the ethereal solution was dried over anhydrous sodium sulphate.

The ether was evaporated under reduced pressure and the residue crystallised from 5:2 carbon tetrachloride - light petroleum 40 - 60.

Yield of pure N carbobenzoxy L serine benzyl ester 3.5 gm. (75%) m.p. 83 - 84°C. $[\alpha]_D^{20} = +6.0^\circ$ in chloroform.

Preparation of N carbobenzoxy L serine benzyl ester phenyl phosphate.

In a 100 ml. conical flask equipped with a magnetic stirrer and cooled in an ice bath were placed 1.2 gm. (0.0057 mole) monophenyl phosphoryl dichloride ; 0.75 gm (0.0057 mole) dry quinoline and 10 mls. dry chloroform. To this was added dropwise with stirring over 3 hours a solution of 1.85 gm. (0.0057 mole) N carbobenzoxy L serine benzyl ester in 10 mls. dry chloroform. After the addition was complete the mixture was stirred in the cold for 30 minutes and overnight at room temperature.

The solution was now concentrated under reduced pressure and at a temperature below 40°C to a volume of approximately 7 mls, and 0.4 gm. pyridine and 0.5 ml. of water were added. Stirring at room temperature was continued overnight.

After concentration of the mixture below 40°C, 4 mls. water and 1.1 gm. potassium carbonate (3 equivalents)were added, after which the aqueous alkaline solution was extracted four times with 10 ml. portions of ether. The aqueous solution was acidified with 2N nitric acid, and the precipitated oil crystallised by scratching. After cooling overnight in the refrigerator the precipitate was filtered, washed with a little

cold water, and dried in vacuo over concentrated sulphuric acid.

Yield of N carbobenzoxy L serine benzyl ester phenyl phosphate 1.6 gm (62%).

After recrystallisation from chloroform/40 - 60 light petroleum it sintered at 136° and melted at 139.5 - 140.5°C.

$[\alpha]_D^{20}$ = + 12.0° in chloroform.

<u>Preparation of N carbobenzoxy L serine benzyl ester phenyl phosphate potassium salt</u>.

To a warm stirred suspension of 1.4 gm. (0.0029 mole) of N carbobenzoxy L serine benzyl ester phenyl phosphate in 10 ml. water, a solution of 0.2 gm. (0.00145 mole) of anhydrous potassium carbonate in the minimum quantity of water necessary to dissolve it was added. After all the solid had dissolved the solution was evaporated to dryness at the pump below 40°C, and the resulting glassy residue was dissolved in absolute alcohol and reprecipitated by the addition of ether and cooling in the refrigerator. The product, a white pasty solid, was filtered and dried in vacuo over concentrated sulphuric acid.

Yield of potassium salt = 1.2 gm. (80%).

m.pt. 164 - 166°C. $[\alpha]_D^{20}$ = - 12.1° in water.

Preparation of N carbobenzoxy L serine benzyl ester phenyl phosphate silver salt.

1.2 gm. (0.0022 mole) of N carbobenzoxy L serine benzyl ester phenyl phosphate potassium salt were dissolved in 5 mls. of water, and 0.5 gm. (0.0029 mole ; 25% excess) silver nitrate in 0.5 mls. water were added. The oily precipitate was allowed to settle in the dark for two days, then the mother liquor was decanted off, the viscous residue washed with a little water, and dried in vacuo over phosphorus pentoxide in the dark.

1.2 gm. (89%) of a glassy material which on grinding became a fawn coloured powder was obtained.

The substance softened on heating at 85°C and decomposed at 185°C.

Reaction of the silver salt of N carbobenzoxy L serine benzyl ester phenyl phosphate with L 1,2 distearoyl 3 iodo glycerol.

0.9 gm. (0.0015 mole) of the above silver salt were dissolved in 15 mls. refluxing dry xylene, and 0.9 gm. (0.0012 mole ) of L 1,2 distearoyl 3 iodo glycerol (see page 71 ) in 5 ml. dry xylene was added, the mixture being vigorously stirred in the dark during the addition and subsequently for 15 minutes.

The solution was allowed to cool, and was then filtered through a pad of "Filtercel" to remove the precipitated

silver iodide. After concentration at the pump at a temperature not exceeding 40°C, the residue was dissolved in ether, washed twice with 1:1 saturated sodium bicarbonate - water solution, then twice with water, and finally dried over sodium sulphate.

After filtration of the sodium sulphate and evaporation of the ether, a solid product was obtained. This was not purified, but used immediately for the next stage.

Hydrogenolysis of above product to L 1,2 distearoyl phosphatidyl L serine.

1 gm. Adam's platinum oxide and 1 gm. palladium black were reduced with hydrogen in 50 mls. glacial acetic acid, then the acetic acid was decanted off, and the product from the silver salt - iodo compound reaction dissolved in 50 ml. glacial acetic acid was added. (The reason for this procedure was to remove traces of alkali present in Adam's platinum oxide catalyst and prevent the formation of phosphatidyl L serine alkali salts). Hydrogenation was recommenced, occasional warming of the flask and its contents being necessary towards the end of the process to dissolve the product which tended to separate out on the catalyst. The total uptake of hydrogen was 385 mls. (Theoretical 150 mls.).

After uptake had ceased, the catalyst was filtered, washed well with chloroform, and the filtrates were concentrated under reduced pressure.

Isolation and purification of L 1,2 distearoyl phosphatidyl L serine.

After concentration of the filtrates from the hydrogenolysis 1.1 gm. solid residue remained. This was triturated at the centrifuge with three 25 ml. portions of boiling ether.

Yield 0.55 gm. (57%).

A portion of the product was recrystallised from 5 ml. of a solvent containing 22 parts chloroform : 1 part glacial acetic acid, ether being added to the warm solution to precipitate the L phosphatidyl serine. After standing in the refrigerator several hours the precipitate was centrifuged and dried in vacuo over potassium hydroxide. The m.pt. was 159 - 161°C with decomposition.

$[\alpha]_D^{19.5} = -19.7^\circ$ ( 15/1 chloroform-glacial acetic acid).

PART II

## The preparation of unsaturated phosphatides.

## Part II The preparation of unsaturated phosphatides.

### The fatty acids of glycerophosphatides.

The majority of naturally occurring glycerophosphatides are associated with long chain fatty acids. These fatty acids are linked by ester grouping to the hydroxyl groups in the glycerol molecule. They are generally random mixtures of saturated (palmitic, stearic) and unsaturated (oleic, linoleic) acids.

There is some evidence that unsaturated acids are mainly linked at the $\alpha'$ position in the glycerol molecule, and the saturated acids at the $\beta$ position, in the case of the naturally occurring lecithins. Hanahan's work (Progress in the Chemistry of Fats and Other Lipids. Vol. IV. Pergamon Press 1957) on the action of Lecithinases (Lecithin hydrolysing enzymes) suggests this, and it is supported by the findings of Bergstrom and Paabo. (Act. Chem. Scand. 8 1486 (1954)), who studied the metabolism of oleic and palmitic acids in rats, using $C^{14}$ labelled acids as tracers.

As a general rule the phosphatides contain a higher proportion of unsaturated fatty acids than of saturated fatty acids.

The preparation of phosphatides from natural sources is a very tedious process, and it is almost impossible to obtain absolutely pure compounds. Thus it is desirable to be able to

synthesise phosphatides of known constitution and structure, for the examination of their biochemical and physiological effects, and for comparison with compounds obtained from natural sources.

Unambiguous methods for the synthesis of phosphatides containing saturated fatty acids have existed for several years, but modifications of the technique to allow unsaturated compounds to be prepared have been made only recently. For example, L 1,2 distearoyl, dipalmitoyl and dimyristoyl lecithins were prepared by Baer and Kates (JACS. 72 942 (1950)), whereas L 1,2 dioleyl lecithin was not obtained until 1956. (Baer, Buchnea and Newcombe JACS. 78 232 (1956)).

Review of the literature on the preparation of unsaturated glycerophosphatides.

Lecithins.

The method developed by Baer, Buchnea and Newcombe (loc. cit.) for the preparation of L 1,2 dioleyl lecithin has already been described in an earlier section of this dissertation.(See page 31 ). It suffers from two disadvantages:-

a) The stages involving acylation of the barium salt and its condensation with trimethylamine require elevated temperatures, which are detrimental to the purity of the product. The

latter operation is carried out in a sealed tube requiring much care.

b) The product obtained is far from pure and it is necessary to chromatograph it on silicic acid to separate it from lyso lecithin, which is the main impurity, and other contaminating substances. Extrusion of the adsorbent column is essential to permit the recovery of the separated L 1,2 dioleyl lecithin.

No mention was made to the production of bis-phosphorylated products in the phosphorylation stage of the synthesis. This was suprising since earlier papers describing methods for the synthesis of saturated lecithins.mentioned that the mono- and bis - phosphates were separated by means of the ethyl acetate soluble reinecke salt of the mono- phosphorylated compound. It seems likely that a certain amount of bis- compound formation does occur in the preparation of the unsaturated lecithins, and this is not separated until the final chromatographic purification.

Although it is a great step forward in the field of synthetic phospholipid chemistry, the method of Baer, Buchnea and Newcombe has serious defects, which need to be overcome before a general application is possible.

No further papers on the preparation of unsaturated lecithins have appeared up to the time of writing, but a method for preparing saturated lecithins which is applicable to

unsaturated lecithins has been described by Tattrie and McArthur (Canad. J. Bioch. Physiol. 35 1165 (1957)). This involves the direct acylation of the readily accessible glyceryl phosphoryl choline (Baer and Kates JACS 70 1394 (1948)) with an acid chloride in dry chloroform. It was again necessary to purify the crude lecithin by chromatographing on silicic acid, and the main impurity was again lysolecithin. A yield of 31% was obtained, this being for the purified lecithin.

Although this method requires chromatographic technique to separate the pure product it has the advantage of simplicity. Its application to the preparation of unsaturated lecithins would be of interest.

Cephalins.

A cephalin containing an unsaturated fatty acid was prepared some ten years ago by Hunter, Roberts and Kester (JACS 70 3244 (1948)). A mixed acid diglyceride was used as the starting material, namely 1,3 eruco-stearin. The method used was that introduced by Rose (JACS 69 1384 (1947)), using phosphorus oxychloride as the phosphorylating agent and reacting this successively with the diglyceride and 2-hydroxyethyl pthalimide. A yield of 25% of a product melting at 163 - 164°C was obtained, after purifying by trituration with aqueous acetone and crystallisation from ethanol.

The general application of this method to the synthesis of

fully unsaturated cephalins was delayed by the lack of a suitable method for the preparation of unsaturated 1,2 diglycerides. This problem has now been solved by Baer and Buchnea (J.B.C. 230 447 (1958)), and the preparation of L 1,2 dioleyl cephalin has been described in the Report on the Symposium on "Phosphoric Esters and related compounds", Cambridge, April 1957, although full experimental details have yet to be published. The reaction scheme was given on page 40 of this dissertation.

The unsaturated 1,2 diglyceride was obtained by acylating the 1-benzyl ether of glycerol with 9,10 dibromostearoyl chloride,(or the 1,2 dioleyl 3-benzyl ether of glycerol can be brominated at low temperature), removing the benzyl group by catalytic hydrogenolysis, and debrominating with activated zinc dust in ether.

The preparation of the cephalin followed the general method c.f. Rose (loc. cit), but it was necessary to purify the product by chromatographing on a silicic acid column. This indicated that the product was not as pure as the saturated cephalins prepared by the same method- these latter were purified by trituration with ether and recrystallisation from ethanol. c.f. Bevan and Malkin J. 2667 (1951).

The above method is elegant in its conception, but is somewhat tedious in its application. The preparation of the

unsaturated 1,2 diglyceride requires care, owing to the lack of crystalline intermediates, these being mainly oils. A further disadvantage is the need to purify the product chromatographically.

Baer and Buchnea (loc. cit.) had attempted the synthesis of this cephalin using mono-phenyl phosphoryl dichloride as a phosphorylating agent, reacting it successively with 1,2 di - (9,10 dibromostearin) and N carbobenzoxy ethanolamine. The phenyl and carbobenzoxy groups were removed by catalytic hydrogenolysis, and the resulting 1,2 di (9,10 dibromo) stearoyl cephalin was treated with zinc dust in various solvents in an attempt to produce the unsaturated compound, but with no success.

Baer and Buchnea (J.B.C. 230 447 (1958)) stated that hydrolysis of the 1,2 diolein they prepared yielded almost pure oleic acid in good yield, indicating that no cis- trans conversion had occurred during the bromination and debromination processes.

Discussion of approaches to the preparation of unsaturated phosphatides.

A considerable amount of work has been carried out by the author in attempts to prepare unsaturated phosphatides. In part this has been successful, and unsaturated cephalins have been prepared as definite compounds. Several possible lines of approach had to be rejected, however, owing to lack of time.

Lecithins.

The preparation of unsaturated lecithins by conventional methods, that is, those used for saturated compounds, cannot be effected for one principal reason viz; it is impossible to remove a phosphate protecting group such as a phenyl or benzyl by catalytic hydrogenolysis without concurrently reducing the unsaturated acid moieties present to the corresponding saturated compounds. Various attempts to do this using poisoned catalysts and other means have failed to achieve the desired result. (c.f. Baer and Buchnea J.B.C. 230 447 (1958)).

In addition to this until recently it was impossible to prepare an unsaturated 1,2 diglyceride, although unsaturated 1,3 diglycerides have been readily available for some time. (Fischer. Ber 53 1621 (1920)).

The various possible ways of overcoming these difficulties can be conveniently considered under four classed. These will be dealt with later on in this dissertation.

Group A. Use of a phosphorylating agent which contains no protecting groups e.g. $POCl_3$, or one in which the protecting groups can be removed without hydrogenolysis e.g. the $-P(=O)-NMe_2$ and $-P(=O)-NHC_6H_5$ protecting groups.

Group B. Use of compounds such as:-

$Cl_2P(=O)-OCH_2CH_2Cl$ and $Cl_2P(=O)-OCH_2CH_2Br$ as phosphorylating

agents. In general there is less bis- compound formation with these than with $POCl_3$.

Group C. Removal of protecting groups (by catalytic hydrogenolysis) prior to acylation of the glycerol moiety with unsaturated acid chlorides. This method, the basis of Baer, Buchnea and Newcombe's work (loc.cit.) always results in partial lyso compound formation.

Group D. Coupling reactions of silver salts with iodides containing unsaturated acid moieties.

Cephalins.

The cephalins present fewer difficulties than the lecithins, both for the saturated and unsaturated compounds. In the case of the saturated cephalins, an elegant synthesis by means of a silver salt - iodo compound reaction has been developed in these laboratories by Baylis, Bevan and Malkin (Report on Biochemical Problems of Lipids. Ghent 1955, Butterworths), and this has been used by the author as described in Part 1 of this dissertation for the preparation of optically active saturated cephalins.

As with the lecithins, the preparation of unsaturated cephalins requires the use of a phosphate protecting group capable of being cleaved without hydrogenation. In the author's experience, the benzyl group $-\overset{\overset{O}{\|}}{\underset{\backslash}{P}}-OCH_2C_6H_5$ is the most

satisfactory in this respect, since a variety of methods exist for the selective removal of the benzyl group from a phosphate molecule (vide infra ).

The use of phosphorus oxychloride in the preparation of unsaturated cephalins has already been described, and this reagent completely solves the problem of protecting groups. A drawback with its use is the tendency to form bis- compounds, and the final products usually require rather more purification than those obtained by other methods.

Discussion of Experimental Work.

Lecithins.

In the previous section the possible approaches to the problem of synthesising unsaturated lecithins were subdivided into four groups. Of these, the first three (i.e. groups A), B) and C) ) will be dealt with now under the heading of direct phosphorylation methods, while group D) will be considered separately in the section on the use of silver salt reactions.

Direct Phosphorylation methods.

The work set out in this account is not in chronological order, but in order of its relative success in achieving the desired result. Some of the material in the later part of the section is included for the sake of completeness only.

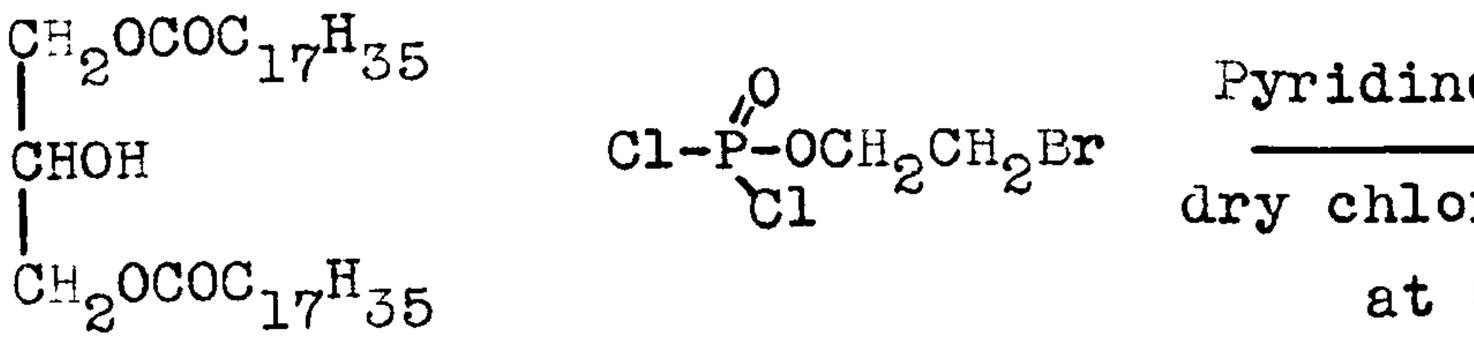
The use of 2-bromo ethyl phosphoryl dichloride as a phosphorylating agent. (Group B.)

2-bromo ethyl phosphoryl dichloride was prepared by gently refluxing a mixture of phosphorus oxychloride and ethylene bromhydrin, followed by fractionation at the water pump. The product was a clear liquid b.pt. 105 - 110°C/10 m.m.

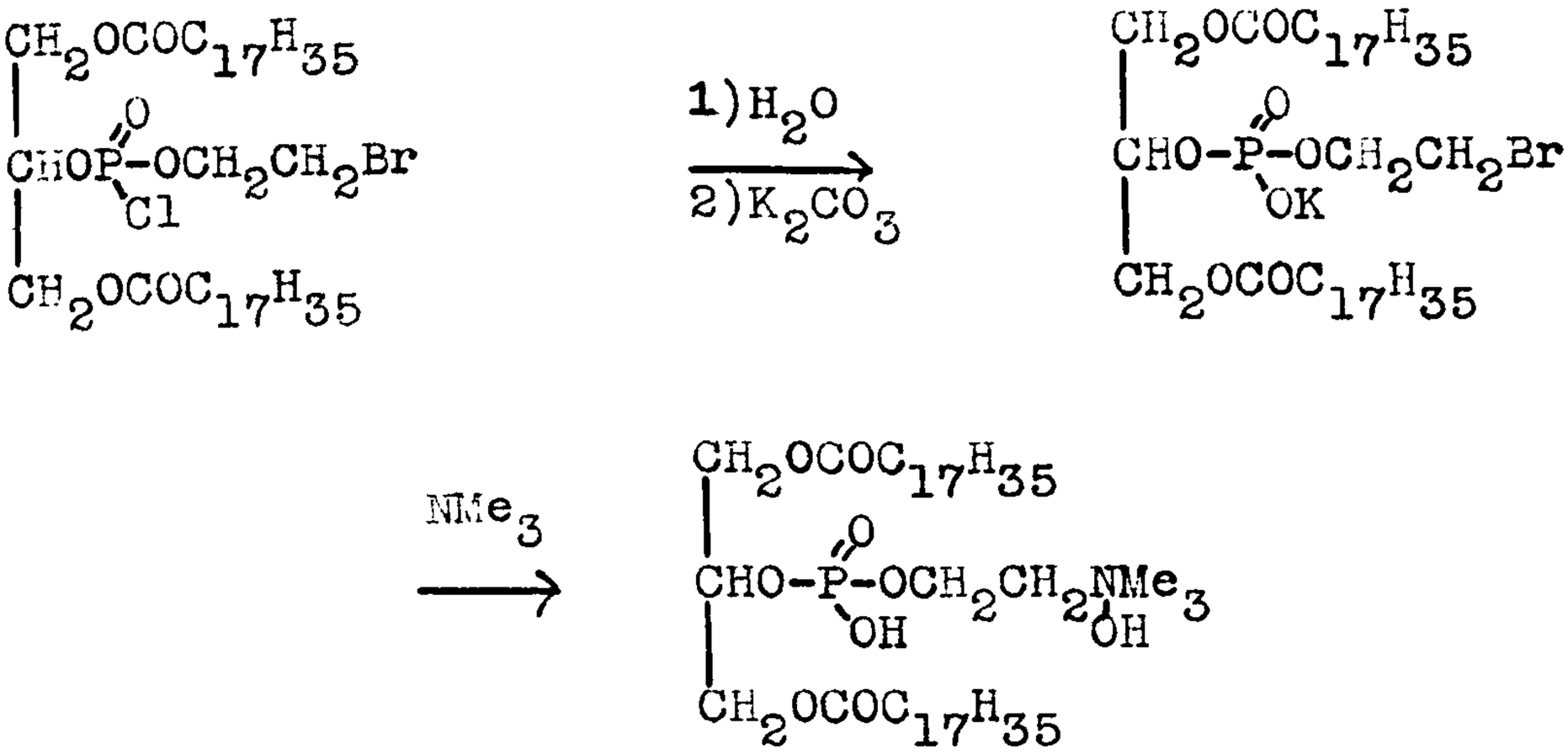
After characterisation by means of its aniline derivative this reagent was used to phosphorylate a 1,3 diglyceride and a 1,2 diglyceride, in different experiments. The conditions in the second case were somewhat modified owing to the low yield of phosphorylated product in the first.

Phosphorylation of 1,3 distearin with 2-bromo ethyl phosphoryl dichloride.

The proposed reaction scheme in this attempt to prepare 1,3 distearoyl lecithin was as follows:-



1,3 Distearin



1,3 Distearoyl lecithin.

The reaction was carried out in dry chloroform at 0-5°C, the 1,3 distearin being added slowly to a stirred mixture of the phosphoryl dichloride and pyridine. When the reaction mixture was worked up, a pasty white solid was obtained, acid to litmus and giving a strong green flame with copper wire, indicating the presence of halogen. After recrystallising this twice from light petroleum 40-60 however, a compound was obtained with a m.pt. 75-77°C alone and undepressed when mixed with 1,3 distearin. It was neutral and gave only a weak halogen test. The X-ray side spacings were photographed (Philips X-ray

tube, copper target, Cu $K_{\alpha}$ radiation wavelength 1.54 Å, Cu $K_{\beta}$ radiation being filtered out by a nickel filter 0.02 m.m. thick) and compared with those obtained from an authentic specimen of 1,3 distearin. They were identical, showing that the compound was 1,3 distearin which had not been phosphorylated.

Although some phosphorylation had probably occurred, the fact that separation of pure starting material by crystallisation was possible suggested that only a small proportion of the starting material had reacted. Evidently the phosphorylating agent was rather unreactive, and the use of chloroform as the reaction medium further reduced its efficiency. In an attempt to overcome this with 1,2 distearin, pyridine was used as the reaction solvent.

Phosphorylation of 1,2 distearin with 2-bromo ethyl phosphoryl dichloride.

The overall scheme for the preparation of 1,2 distearoyl lecithin was the same as in the previously described experiment, except that 1,2 distearin was used instead of 1,3 distearin. The solid diglyceride was added portionwise with stirring to the phosphoryl dichloride in a large excess of dry pyridine. The precipitation of pyridine hydrochloride indicated that the reaction was proceeding.

From this attempt a product was obtained which after three recrystallisations from light petroleum 40-60 had a constant

m.pt. of 67-9°C. It was acid to litmus and contained halogen. Analysis figures indicated however that it was approximately a 50/50 mixture of required product and starting material. Time did not permit the further investigation of this method.

In view of the fact that partial phosphorylation had occurred and that the condensation with trimethylamine could probably be carried out under fairly mild conditions (for a bromide), this method would perhaps be worth more prolonged study. By modifying the conditions such as temperature and concentration of reactants the yield of phosphorylated intermediate could probably be improved. Dr. T. H. Bevan, of these laboratories, has recently found that concentration is a very critical factor in preparing p-toluene sulphonyl derivatives of various alcohols, increasing concentration resulting in improved yields, and sometimes causing a reaction to proceed where previous attempts with lower concentrations had failed.

There is also the possibility that the use of a more active base such as 2,6 lutidine instead of pyridine would expedite the reaction.

<u>The use of 2-chloroethyl phosphoryl dichloride as a phosphorylating agent</u>. (Group B.)

Prior to the work described in the previous section, 2-chloroethyl phosphoryl dichloride was used in an attempt to

prepare a lecithin. The phosphorylation proceeded more readily in this case, but it was not possible to condense the resulting intermediate with trimethylamine. The proposed reaction scheme closely resembled that for 2-bromoethyl phosphoryl dichloride, except that a long chain alcohol (octadecyl) was used instead of a diglyceride, owing to the unavailability of the latter at the time. This did not affect the general scheme.




Lecithin analogue of Octadecyl alcohol.

2-chloroethyl phosphoryl dichloride was prepared according to the method of Plimmer and Burch (Bioch. J. 31 398 (1937)) by refluxing together equimolecular amounts of ethylene chlorhydrin and phosphorus oxychloride for 1 hour, followed by distillation under reduced pressure. b.pt. 103°C/12m.m. of mercury. A yield of 62% was obtained.

Phosphorylation of octadecyl alcohol with 2-chloroethyl phosphoryl dichloride.

The octadecyl alcohol was dissolved in dry chloroform and the solution was added dropwise with stirring to an ice cold mixture of 2-chloroethyl phosphoryl dichloride, pyridine and chloroform. After overnight stirring at room temperature, water was added to decompose the intermediate mono-chloride, and the reaction mixture was concentrated and the product obtained. It was a white pasty solid m.pt. 45-51°C, but after three recrystallisations from 40-60 light petroleum (alcohol was also tried less successfully) the m.pt. was constant at 58-60°C. Analysis figures were correct for the required product, and it was acid to litmus and gave a strong green flame on copper wire (halogen present).

The sodium salt of the above compound was prepared by neutralising an alcoholic solution of the octadecyl 2-chloro-ethyl phosphate with sodium carbonate solution, concentrating

to dryness and extracting the residue with absolute alcohol.

An attempt was made to exchange the chlorine atom present in this sodium salt for an iodine atom by refluxing the sodium octadecyl 2-chloroethyl phosphate with excess sodium iodide in methyl ethyl ketone, but decomposition occurred.

This was attempted because it seemed probable that the condensation of the iodide with trimethylamine would proceed very much more readily than the chloride, and it was anticipated that the use of sealed tubes for the condensation reaction could be avoided in this way.

Another portion of the sodium salt was dissolved in dry benzene and treated with four equivalents of anhydrous trimethylamine at 60°C for four days, the reaction being carried out in a sealed tube. The product obtained however, was unchanged starting material, and a sample of the free acid prepared from the sodium salt had an undepressed melting point of 56-59°C when mixed with a sample of the original 2-chloroethyl octadecyl phosphate.

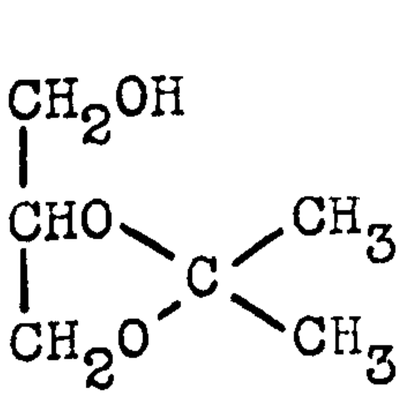
It was possible that the condensation reaction would be successful if a higher concentration of trimethylamine was used, but owing to the difficulties of doing this and the possibility of using 2-bromoethyl phosphoryl dichloride, the product from which would condense more readily, the approach was abandoned.

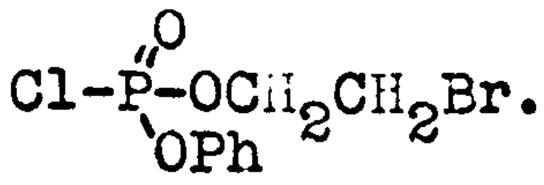
Grun and Kade claimed to have prepared lecithins by this

method as long ago as 1912 (Grun and Kade Ber 45 3367 (1912)), but it is doubtful in the light of modern knowledge whether their products were true lecithins. They used phosphorus pentoxide, ethylene chlorhydrin and a diglyceride, and treated the product with trimethylamine. A mixture of the trimethylamine salt of the acid and "free lecithin" was obtained. This "free lecithin" was probably mainly the choline salt of the corresponding phosphatidic acid.

Attempted preparation of monophenyl 2-bromoethyl phosphoryl monochloride. (Group C.)

A considerable reduction in the work necessary in the application of the method of Baer, Buchnea and Newcombe (loc.cit) could be effected if monophenyl 2-bromoethyl phosphoryl monochloride was available for the first phosphorylation process. This could be reacted directly with acetone glycerol, and no possibility of bis compound formation exists.

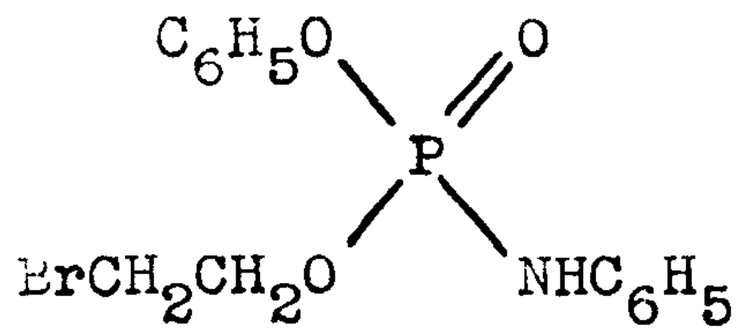




Pyridine.

For this reason it was decided to attempt to prepare this reagent. Two completely different approaches were tried. In the first ethylene bromhydrin and monophenyl phosphoryl dichloride

were refluxed together in dry benzene for 5½ hours. On removal of the solvent and distillation in vacuo a very small quantity of liquid b. pt. 80-110°C was obtained, which yielded an aniline derivative m.pt. 128°C. This derivative analysed correctly for that of monophenyl 2-bromoethyl phosphoryl monochloride. i.e.




On raising the distillation temperature above 110°C decomposition occurred.

Since the yield for this reaction was impracticably small an alternative method of preparation was investigated, namely, phosphorylation of ethylene bromohydrin with monophenyl phosphoryl dichloride in the presence of quinoline.

The ethylene bromhydrin was added dropwise to a vigorously stirred mixture of monophenyl phosphoryl dichloride and quinoline, the temperature being maintained at -15° to -20°C. The product was extracted with dry benzene, quinoline hydrochloride being removed by rapid filtration, and the solvents were evaporated under reduced pressure.

An attempt to distil the residue in vacuo was unsuccessful,

and this ...

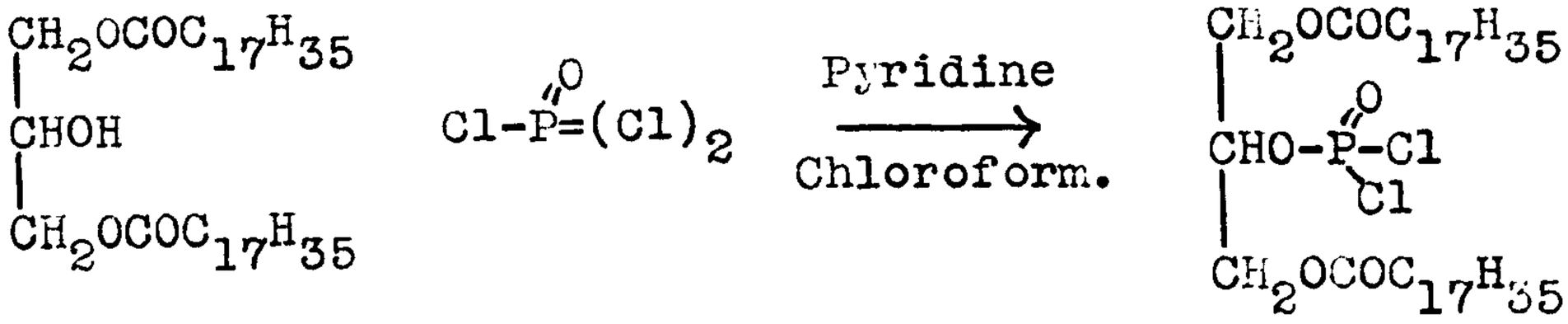
was therefore converted into the aniline derivative. Analysis figures indicated that monophenyl phosphoryl dichloride predominated in the mixture of substances present.

As a result of these two unsuccessful attempts to prepare monophenyl 2-bromoethyl phosphoryl monochloride, together with a further attempt made by Dr. T. H. Bevan of these laboratories, it was decided to abandon this approach.

The use of phosphorus oxychloride as a phosphorylating agent (Group A.)

In the theoretical section of Part II of this dissertation the use of phosphorus oxychloride in the preparation of saturated and unsaturated cephalins has been described. Although a certain amount of bis compound formation accompanied its use, in general it provided a satisfactory way for the preparation of the saturated cephalins, and it was the reagent used in the first reliable synthesis of these compounds. For the above reasons it was decided to attempt to prepare a saturated lecithin by using phosphorus oxychloride.

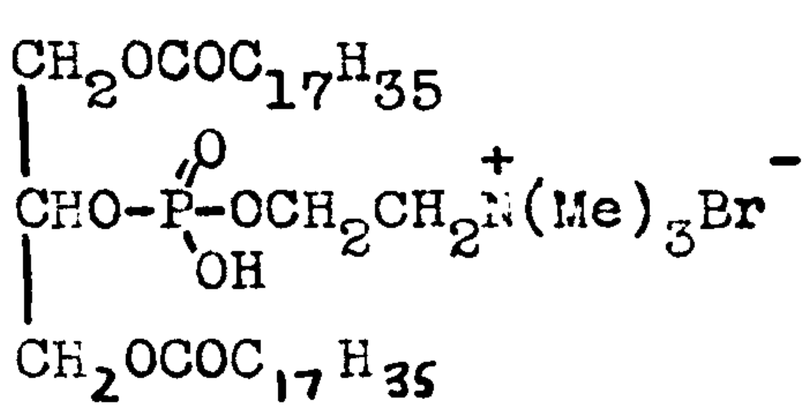
Using 1,3 distearin and choline bromide as starting materials the proposed reaction scheme, which has an overall similarity to that used for the cephalins, was as follows:-



1,3 Distearin

1) $HOCH_2CH_2\overset{+}{N}(Me)_3Br^-$ Choline bromide.

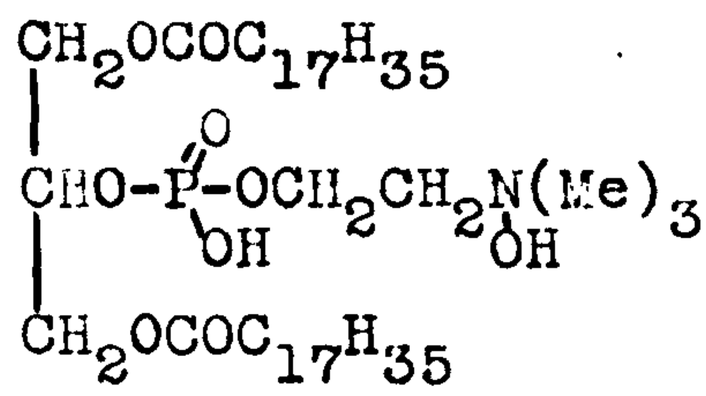
2) $H_2O$



1) Ammonium reineckate.

2) $Ag_2SO_4$

3) $BaCO_3$



1,3 Distearoyl lecithin.

Choline bromide was selected for the following reasons.

a) It was much more soluble in pyridine than choline chloride, and hence more likely to react.

b) It was less hygroscopic than choline chloride and was in general easier to handle.

c) It was more stable than choline iodide - trial small scale experiments indicated that choline iodide decomposed under the conditions of this reaction.

Attempted preparation of 1,3 distearoyl lecithin.

The 1,3 distearin was dissolved in dry chloroform and added dropwise to an ice cold stirred mixture of phosphorus oxychloride, pyridine and chloroform. After completion of the initial phosphorylation reaction, the choline bromide was added slowly dissolved in a pyridine -NN' dimethyl formamide mixture. After stirring overnight water was added to hydrolyse the remaining chlorine atom , and after further stirring the solution was worked up. A white amorphous powder was obtained m.pt. 175-6$^{o}$C after two recrystallisations from chloroform/ethanol. It failed to give a cadmium chloride complex or a reineckate precipitate, and the nitrogen and phosphorus analysis values were approximately one third of the theoretical. The nitrogen/ phosphorus ratio was 1:1.

It was thought that some sort of complex compound formation took place in this reaction, the precise nature of which was a matter of pure speculation. At the time the author was actively engaged on other work and did not have an opportunity to investigate this as fully as the complexity of the problem required. It was definitely established that the method did not yield the required lecithin.

Silver Salt Reaction Methods. (Group D.)

Although several references to the preparation of cephalins, phosphatidyl serine, phosphatidic acids and alkyl phosphates through the use of silver salt-iodo compound reactions are to be found in the literature in recent years, as yet no reliable synthesis of the lecithins has been achieved in this way. Much of the work relating to the afore mentioned classes of compounds was carried out in these laboratories, and it was found that in nearly every case the silver salt-iodo compound method gave better yields and purer compounds than were obtainable by other procedures.

For the above reasons the author investigated the preparation and use of certain silver salts in attempts to synthesise lecithins, with the additional objective in view of obtaining the unsaturated compounds.

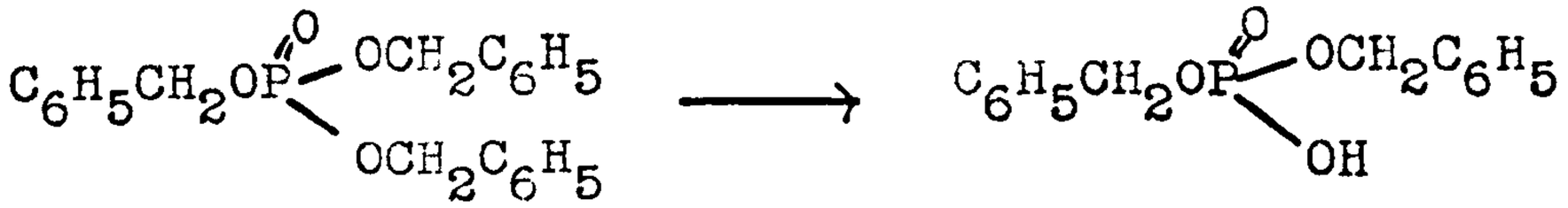
As with the discussion of the work done on direct phosphorylation methods, the following account is not presented in the order in which the work was carried out, but in order of the relative importance in achieving a solution to the problem.

The use of benzyl and p-nitrobenzyl phosphate silver salts.

There are several methods of removing a benzyl or p-nitrobenzyl protecting group from a phosphate molecule. The best known is that of catalytic hydrogenation using a palladium

catalyst, and this method finds wide application when it is desired to remove all protecting groups simultaneously.

In the field of phospholipid synthesis however, there are many occasions when it is desirable to remove only one protecting group at a time. For example during the preparation dibenzyl hydrogen phosphate from tribenzyl phosphate, such a procedure would be necessary:-



This can be achieved by several different methods viz:-

a) With various tertiary bases such as pyridine, quinoline, morpholine and N methyl morpholine. Better yields are obtainable using the latter. (Todd et al J 815 (1949) ; ibid 2023 (1950)).

b) With various alkali metal and alkaline earth salts in certain solvents. e.g. lithium chloride and potassium acetate in ethoxyethanol (Todd et al J. 2030 (1950) ; 2381 (1954)), and sodium iodide and barium iodide in acetone (Zervas and Dilaris JACS 77 5354 (1955)). Of these the author found the latter ~~method~~ most effective, and this is supported by the findings of Verkade et al (Proc. K. Ned. Akad. Wet. 60 B. 308 (1957)).

c) With phenol. (Kenner and Mather J. 3524 (1956)). This process is less selective and a certain amount of total

debenzylation occurs.

d) By irradiation with ultra-violet light. (Arris, Baddiley, Buchanan and Thain J. 4968 (1956)). This method, which has been used only for very small quantaties of material, again results in complete removal of the benzyl groups occurring for part of the material.

The method of Zervas and Dilaris involving the use of sodium iodide in acetone was found to be the most suitable method for monodebenzylation and mono de p-nitrobenzylation of phosphoric esters.

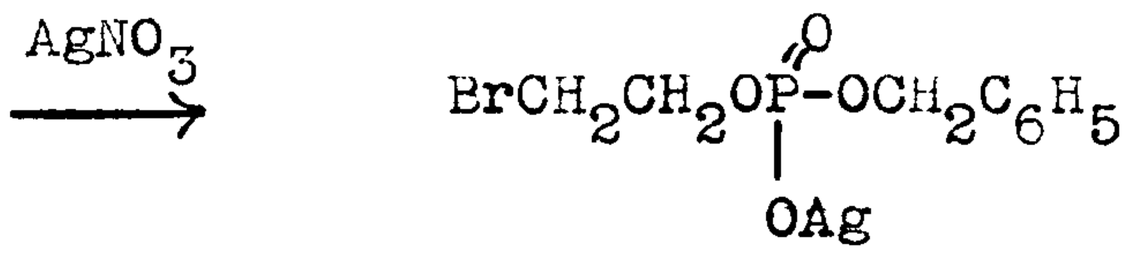
Use of Bromo-Iodo-ethylene.

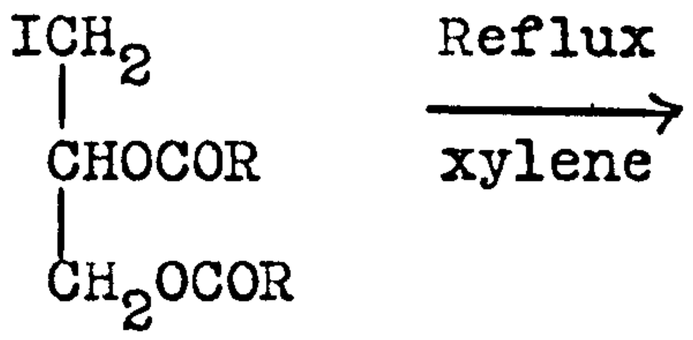
A number of different reaction schemes can be devised involving the use of benzyl phosphate silver salts for the preparation of lecithins. For example:-

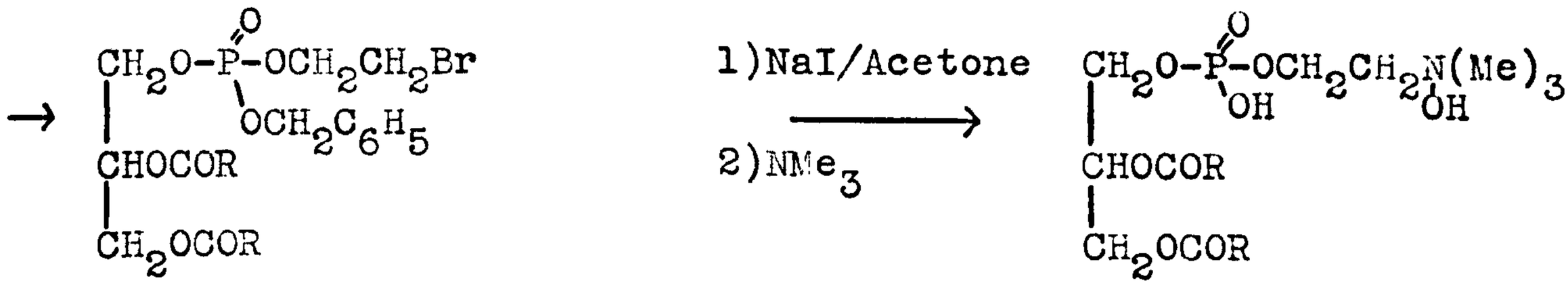
$$BrCH_2CH_2I \qquad AgO\text{-}P(=O)(OCH_2C_6H_5)_2 \longrightarrow$$

$$\rightarrow \quad BrCH_2CH_2OP(=O)(OCH_2C_6H_5)_2 \xrightarrow[\text{Acetone}]{NaI} BrCH_2CH_2OP(=O)(ONa)(OCH_2C_6H_5)$$

P.T.O.

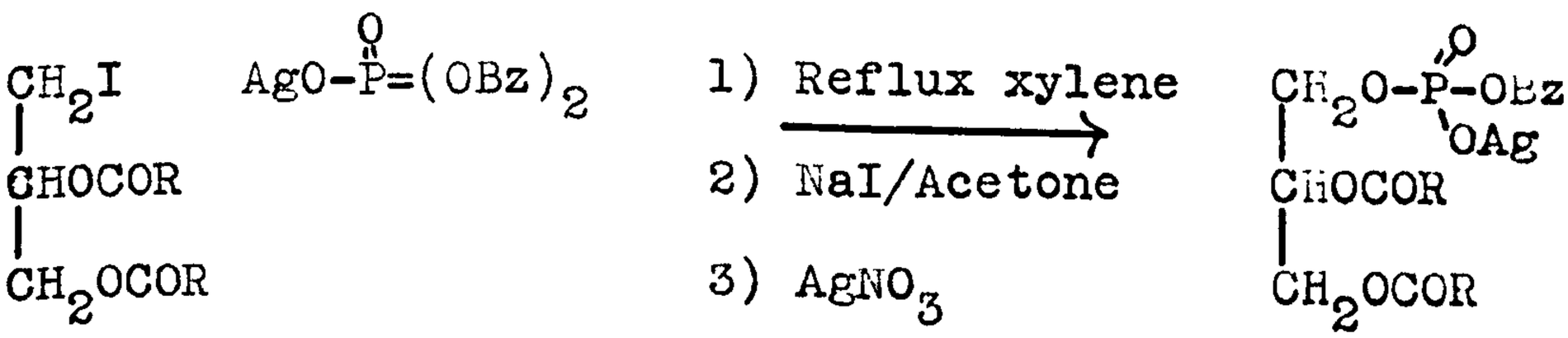






1,2 Diacyl lecithin.

A slight modification of this method would be to react the dibenzyl phosphate silver salt with the 1,2 diacyl glycerol iodohydrin in the first stage, viz:-



the later stages of the synthesis being identical with the first.

The main difficulty encountered with this methodwas the preparation of bromo-iodo ethylene. An attempt to do this by preparing the p-toluene sulphonyl derivative of ethylene bromhydrin and subsequent exchange with sodium iodide in acetone

was not successful, probably because the tosylation reaction did not proceed satisfactorily. This has been found to be the case with many halogen containing compounds. Possibly an increase in reactant concentration would expedite the process. There is also the possibility that bromo iodo ethylene could be prepared by the Arbuznov reaction, that is, the conversion of a hydroxyl group to an iodine atom using the triphenyl phosphite methiodide reagent. (c.f. Preparation of glycerol 1-iodohydrin by Bevan, Malkin and Smith J. 1383 (1955)).

Provided a satisfactory method for the preparation of bromo-iodo ethylene is available, this method shows considerable promise as a route to the lecithins. The modification in which the dibenzyl phosphate silver salt is reacted with the 1,2 diacyl iodohydrin would probably be easier to work through, since the intermediates would more likely be crystalline compounds. The possibility of silver - bromine interaction would also be reduced.

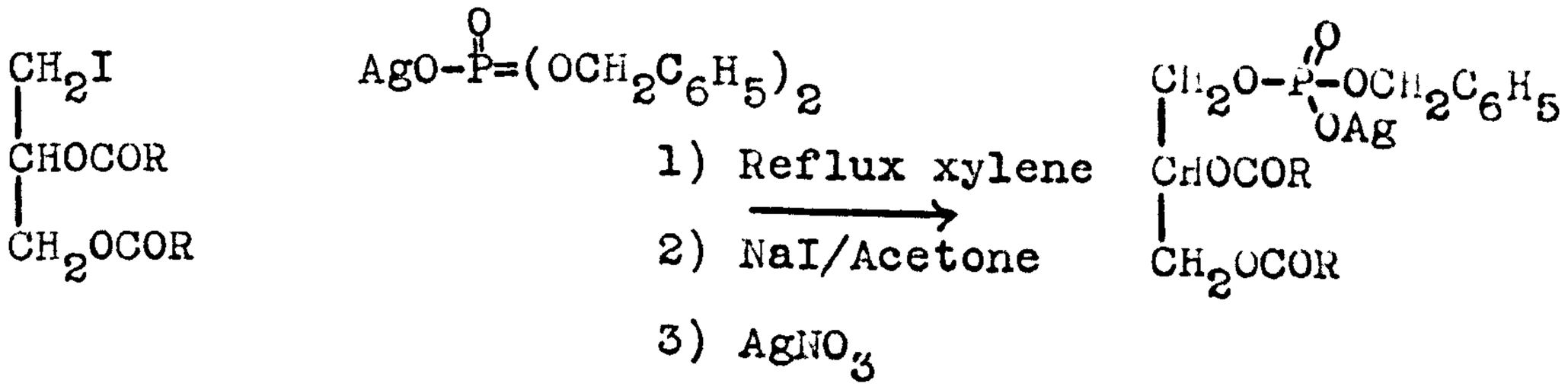
The condensation of the bromine atom with trimethylamine would be expected to proceed fairly readily, and little difficulty should be experienced at this stage.

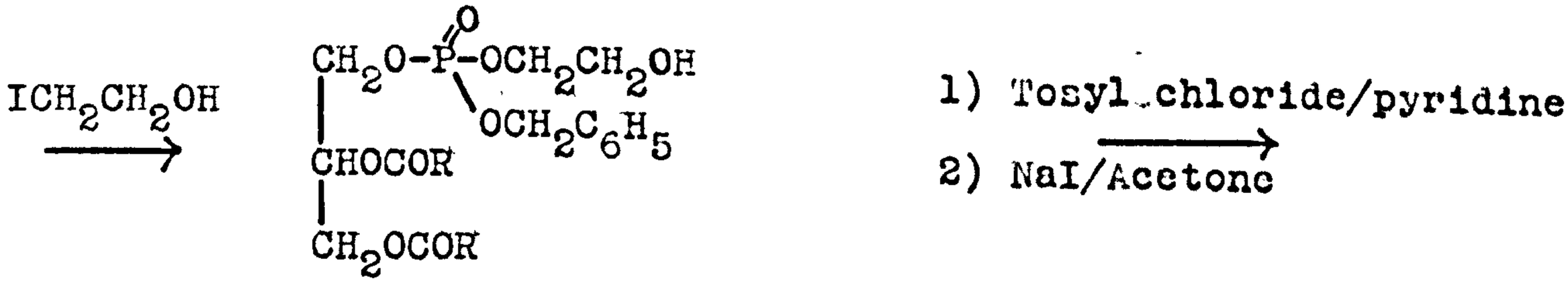
Some of the early work carried out by the author involved the use of p-nitrobenzyl protecting groups. Although the compounds obtained were generally crystalline solids and easier to work with on that account, the preparation of the starting

materials such as the di-p-nitrobenzyl phosphate silver salt was rendered more difficult by the reduced reactivity of p-nitrobenzyl compounds as opposed to benzyl compounds. For this reason the use of these substituted benzyl groups was discontinued.

The use of ethylene iodohydrin.

Although no experimental work has been carried out in support of the use of ethylene iodohydrin in the synthesis of lecithins, the possibility exists that it could be used as follows:-

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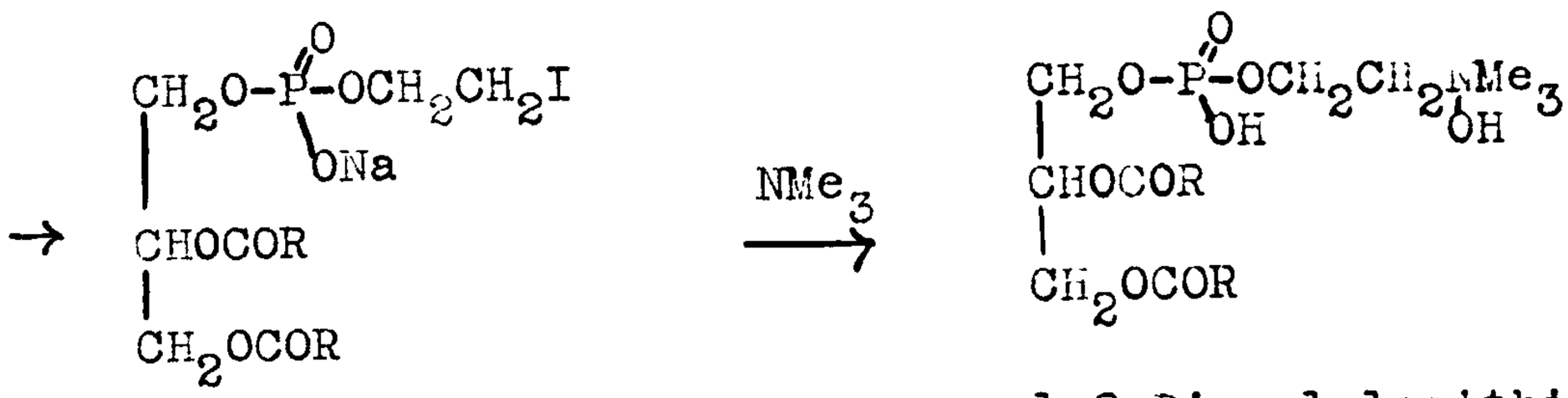
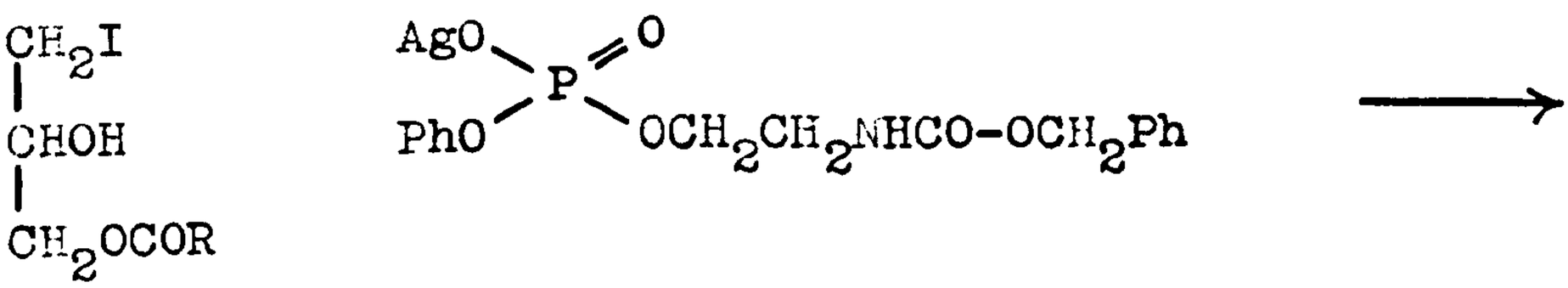


1,2 Diacyl lecithin.

Two main difficulties could arise using this scheme.

a) The silver salt reaction with ethylene iodohydrin may be adversely affected by the presence of a free hydroxyl group in the ethylene iodohydrin molecule, as in the case of certain other silver salt reactions e.g.



This reaction, which would be used for the preparation of "lyso" cephalins, fails completely, presumably because of the presence of the free hydroxyl group adjacent to the iodine atom.

b) The tosylation of the 1,2 diacyl glyceryl mono benzyl 2-hydroxy ethyl phosphate intermediate compound could be troublesome, owing to steric hindrance and other factors.

For the above reasons it is felt that this method is not altogether promising, although it is worth attempting if the synthesis using bromo-iodo ethylene failed.

The scope of utilisation of benzyl silver salts in lecithin

synthesis is considerable, although perhaps not so wide as in the case of the cephalins. Much work remains to be done in this field.

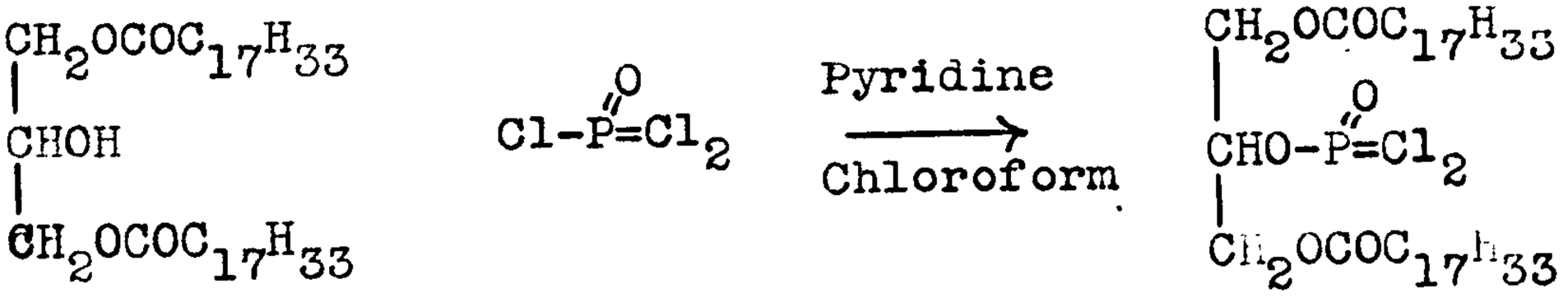
Cephalins.

Methods for preparing unsaturated cephalins have been developed which make use of direct phosphorylation and also silver salt-iodo compound reactions. The direct phosphorylation methods will be considered first, as in the case of the lecithins.

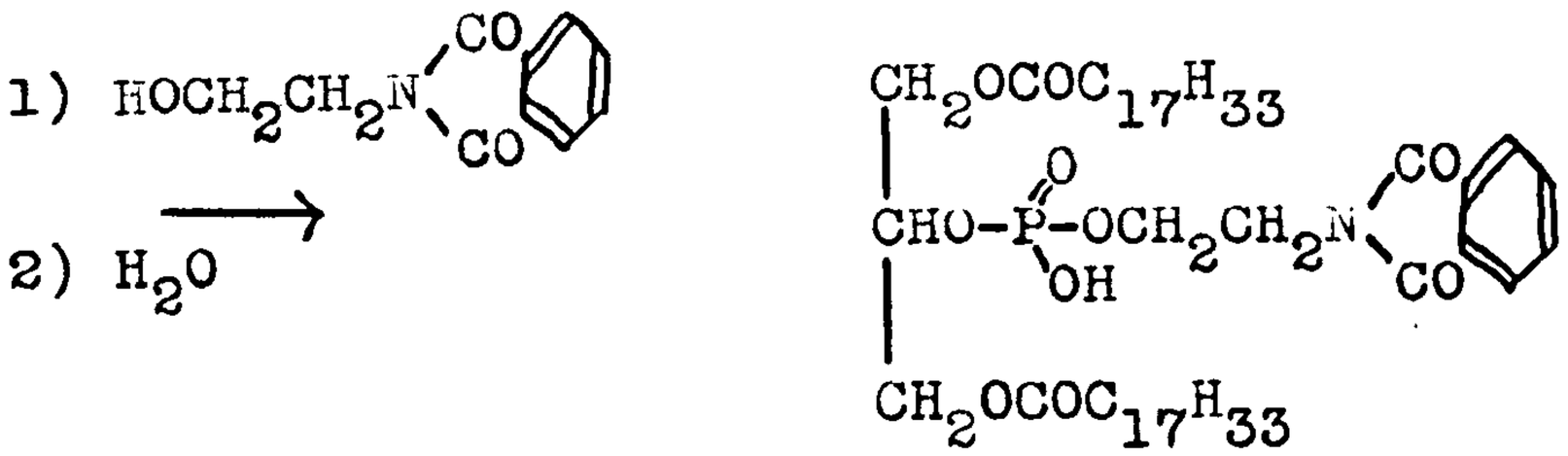
Direct Phosphorylation Methods

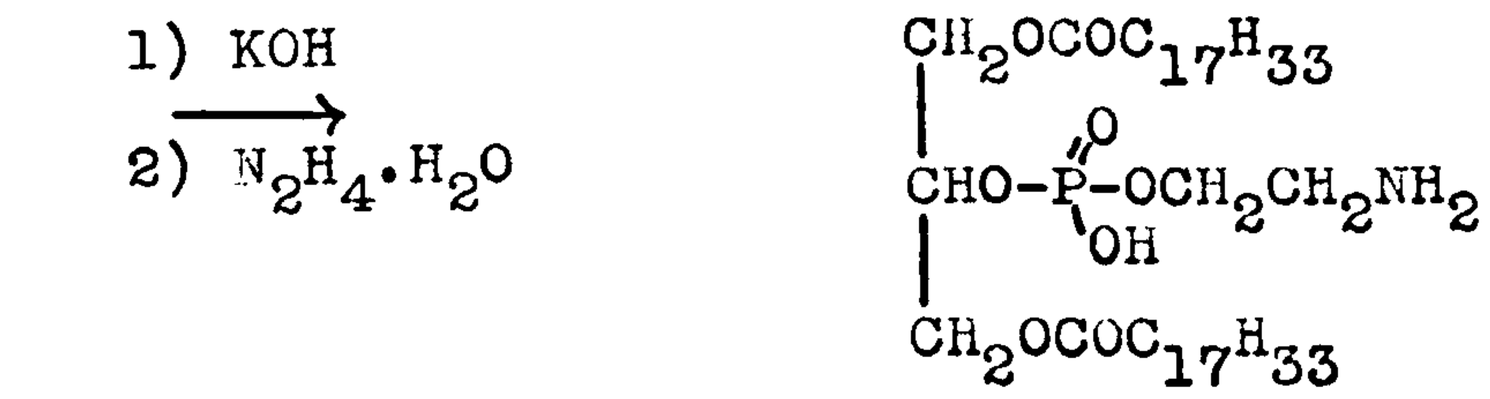
Preparation of 1,3 dioleyl cephalin using phosphorus oxychloride as a phosphorylating agent.

The first authentic synthesis of a saturated cephalin by Rose (loc.cit.) used phosphorus oxychloride as the phosphorylating agent and a diglyceride as the starting material. The author has now extended this to the preparation of 1,3 dioleyl cephalin using 1,3 diolein. (Prepared by Dr. D. B. Smith, of these laboratories.). The overall scheme was as follows:-



1,3 Diolein.





1,3 Dioleyl cephalin.

The 1,3 diolein dissolved in dry chloroform was added dropwise to a stirred mixture of dry chloroform, phosphorus oxychloride and pyridine at a temperature between 10-15°C. Subsequently two equivalents of 2-hydroxyethyl pthalimide in dry chloroform were added. When the reaction was complete solvents were removed by high vacuum evaporation, and the intermediate pthalyl compound was extracted from the residue with boiling ether.

This acidic intermediate was dissolved in methoxy ethanol,

neutralised to bromo-thymol blue (pH 6 ) with 0.5N KOH, and the theoretical quantity of 50% hydrazine hydrate solution was added. After gently refluxing for 1½ hours the solution was allowed to cool and the mother liquor was decanted off from the oily precipitate of free cephalin. The latter was purified by recrystallising from ethanol, yielding a white solid which softened at 110° and melted at 173°C. The substance gave a positive ninhydrin reaction, indicating the presence of an amino group ($-NH_2$), and the analysis figures obtained were in good agreement with the theoretical values.

The 1,3 dioleyl cephalin was soluble in ether, benzene, chloroform, 40-60 light petroleum and hexane. It was only sparingly soluble in acetone and ethanol, the latter being used as a crystallising solvent. It was necessary to stand a fairly dilute solution of the cephalin in ethanol in the refrigerator for several days before crystals were obtained.

The 1,3 dioleyl cephalin was chromatographed on silica impregnated paper, (Prepared by the method of Lea and Rhodes. Biochem. J. 60 353 (1955) ) and samples of various saturated L 1,2 diacyl cephalins were run at the same time. The developing solvent ~~used~~ consisted of an 80/20 mixture of ethanol free chloroform - methanol, and the paper was sprayed with a 0.5% w/v solution of ninhydrin in butanol and heated to 95° C for 5 minutes to reveal the spots.

In all cases the 1,3 dioleyl cephalin gave strong spots, and the Rf values were of the same order as those of the saturated cephalins. They differed widely however from those obtained by Lea and Rhodes - these workers quoted Rf values for saturated cephalins in the region of 0.8, whereas the author in all cases obtained a band in the solvent front (Rf = 1) and a spot at Rf 0.1. This was probably due to the fact that Whatman 3 mm paper was used instead of the faster running Whatman 3 paper, as used by Lea and Rhodes.

Catalytic hydrogenation of a small sample of the 1,3 dioleyl cephalin yielded 1,3 distearoyl cephalin as a white, ether insoluble solid m.pt. 196°C. Analysis figures for this compound also corresponded with the theoretical results.

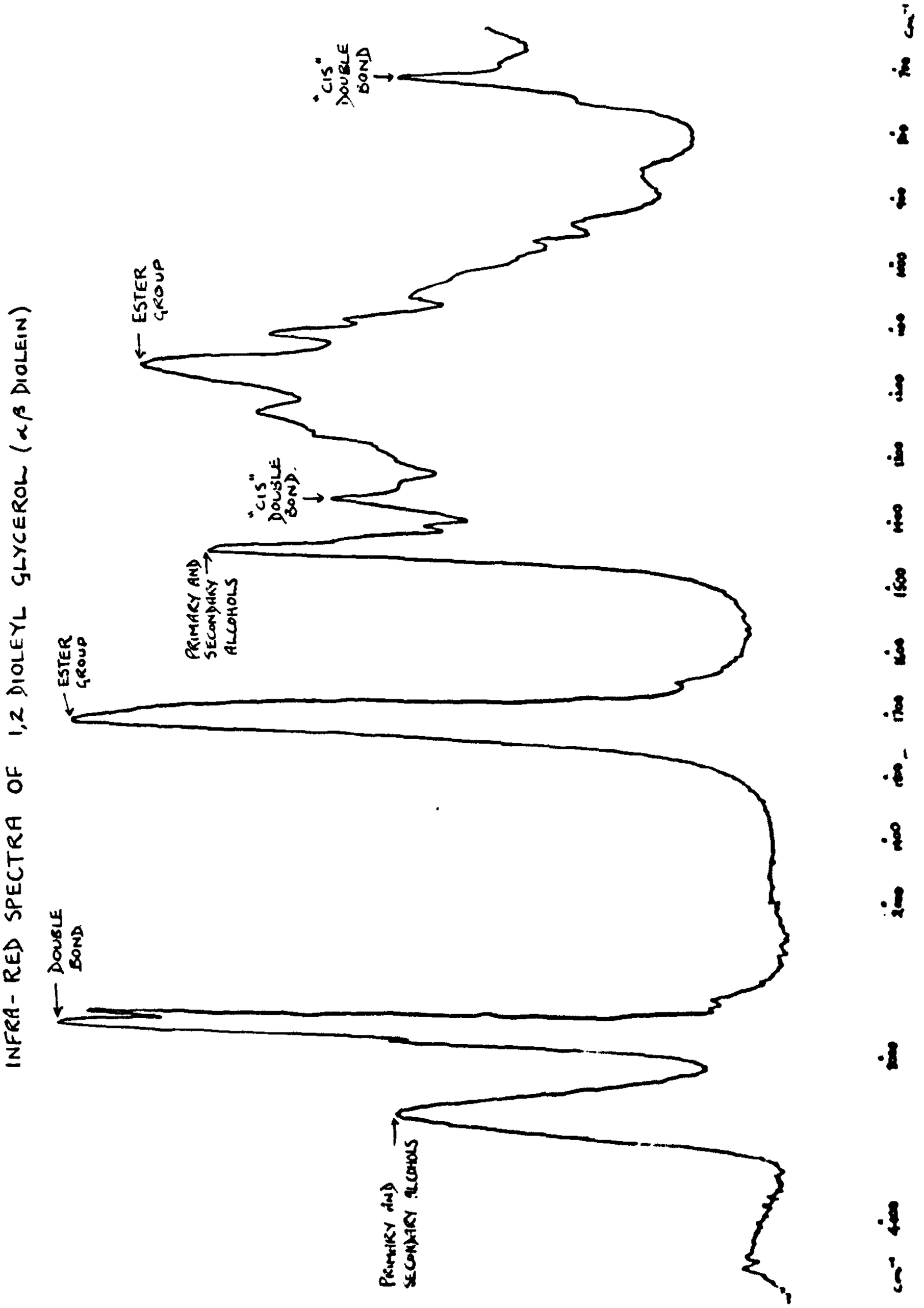
The Preparation of 1,2 dioleyl cephalin.

Preparation of 1,2 diolein.

1,2 diolein was prepared by a method similar to that very recently described by Baer and Buchnea J.B.C. 230 447 (1958). The 1,2 diolein so obtained was purified by recrystallising from hexane at -35°C, when it separated out readily as a solid. It was characterised by reducing it in n-hexane with palladium black to 1,2 distearin m.pt. 70-72°C.

Analysis figures on the 1,3 diolein were in close agreement with the theoretical values, and the infra red spectra gave

PLATE II


INFRA-RED SPECTRA OF 1,2 DIOLEYL GLYCEROL (α,β DIOLEIN)
DOUBLE BOND
ESTER GROUP
PRIMARY AND SECONDARY ALCOHOLS
"CIS" DOUBLE BOND
ESTER GROUP
PRIMARY AND SECONDARY ALCOHOLS
"CIS" DOUBLE BOND
PRIMARY AND SECONDARY ALCOHOLS
100
90
80
70
60
50
40
30
20
10
0

peaks at 1350 $cm^{-1}$ and 720 $cm^{-1}$ (Plate II page 117) indicating the presence of cis- double bonds. No peaks corresponding to trans- double bonds were observed, showing that no elaidinisation had occurred.

This 1,2 diolein was used as the starting material in the synthesis of 1,2 dioleyl cephalin.

Phosphorylation of 1,2 diolein with 2-pthalimido ethyl dichlor-phosphonate.

A recent paper by Hirt and Berchtold (Helv. Chim. Acta. 40 1928 (1957)) described the use of 2-pthalimido ethyl dichlor-phosphonate as a phosphorylating agent for the preparation of various cephalins and alkyl analogues of cephalin. The dichlor-phosphonate, a crystalline solid, was prepared by refluxing phosphorus oxychloride and 2-hydroxyethyl pthalimide together in dry benzene for 4 hours.

Bis-compound formation during the phosphorylation stage should be greatly reduced using this reagent. Consequently it seemed desirable to attempt to synthesise 1,2 dioleyl cephalin by this route. Subsequent comparison with the work on 1,3 dioleyl cephalin indicated however that little advantage was gained by its use.

A chloroform solution of 1,2 diolein was added dropwise to a stirred ice cold mixture of two equivalents of 2 pthalimido

dichlor-phosphonate, dry pyridine and dry chloroform, and the mixture was stirred for two days at room temperature. After addition of water and further stirring, the solvents were distilled off in vacuo, and the residue extracted with boiling ether.

After evaporation of the ether the viscous acid residue was dissolved in methoxy ethanol and neutralised with 0.5N potassium hydroxide to pH 6. The theoretical weight of hydrazine hydrate was then added, and the solution was stirred in the cold overnight. An oily precipitate appeared, which was separated from the mother liquor and dissolved in ether. After washing with water, drying with sodium sulphate and evaporating off the ether a yellow oil was obtained. This gave a positive ninhydrin test and gave spots on a chromatogram with similar Rf values to those obtained with 1,3 dioleyl cephalin, but it proved impossible to recrystallise the substance from ethanol or any other solvent. It seemed that this sample of 1,2 dioleyl cephalin was less pure than the corresponding 1,3 dioleyl cephalin, although no apparent reason for this discrepancy can be suggested. Possibly it could be more readily purified by chromatographing on a silicic acid column, as Baer and Buchnea have reported.

The possibility existed that removal of the pthalyl group was incomplete. In an attempt to rectify this a sample was

refluxed with excess hydrazine hydrate in methoxy ethanol for 1½ hours. On cooling a white solid m.p. 112°C was obtained, which proved to be an impure sample of stearoyl hydrazide, produced by alkaline hydrolysis of the oleic acid residues and reduction of the latter with hydrazine to stearic acid.

Analysis. Found. C 72.4 H 13.8 N 7.6

$C_{18}H_{38}ON_2$ requires C 72.4 H 12.7 N 9.4

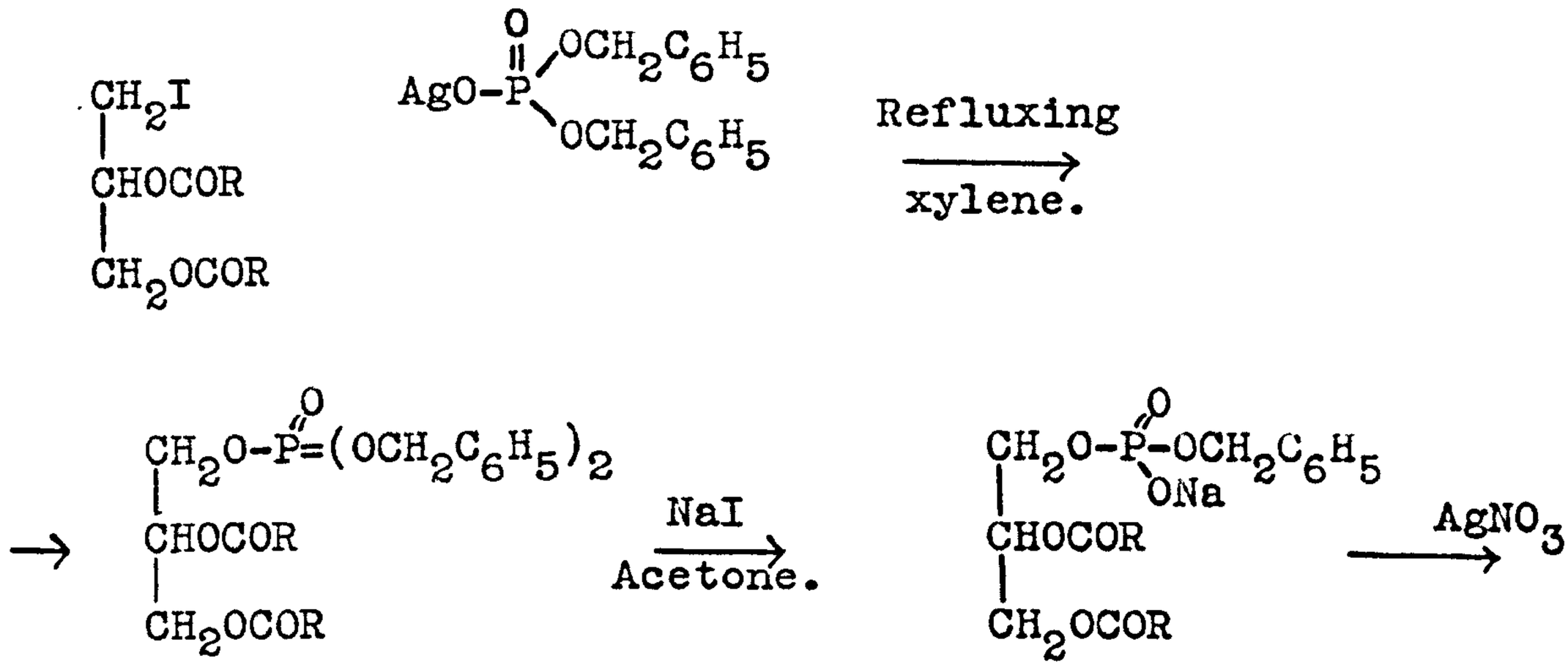
$C_{17}H_{35}CO\text{-}NH\text{-}NH_2$

Silver Salt Reaction Methods.

Preparation of 1,2 distearoyl cephalin starting from Dibenzyl phosphate(silver salt.).

Several different methods for the selective removal of one benzyl group from a benzyl phosphate ester have been described, and two possible routes for the synthesis of unsaturated lecithins using this procedure have been proposed. Slight modifications permit the preparation of 1,2 diacyl cephalins to be achieved, using the following reaction scheme:-

P.T.O.




1,2 Diacyl cephalin.

1,2 distearoyl cephalin has been prepared satisfactorily by this method. The intermediate compounds obtained were crystalline solids with definite melting points, and analysis figures corresponded closely with the theoretical values.

The 1,2 distearoyl glycerol iodohydrin was reacted with silver dibenzyl phosphate in dry xylene, and the product was monodebenzylated by the method of Zervas and Dilaris (loc.cit.) by refluxing with sodium iodide and acetone. The resulting 1,2 distearoyl glyceryl monobenzyl sodium phosphate was converted to the corresponding silver salt with aqueous silver nitrate, and this silver salt, after rigorous drying, was reacted with 2-iodo ethyl pthalimide in refluxing dry xylene. Debenzylation of this substance as before yielded the sodium salt of 1,2 distearoyl glyceryl 2-pthalimido ethyl phosphate, and this was converted to the 1,2 distearoyl cephalin by refluxing with 50% w/v hydrazine hydrate solution in methoxy ethanol. An overall yield of 28% was obtained for the whole series of reactions, and the product had a m.pt. of 195°C. It analysed correctly for 1,2 distearoyl. cephalin.

Extension of this method to the preparation of 1,2 dioleyl cephalin, although theoretically possible, was rather more difficult than anticipated. The intermediate compounds in this case were almost invariably viscous oils, insoluble or nearly insoluble in polar solvents, for example water and ethanol,

and readily soluble in organic solvents such as ether and benzene. In several cases special techniques had to be adopted for the isolation of these intermediate compounds.

The synthesis was satisfactory until the final stage, which was the treatment with 50% w/v hydrazine hydrate solution. At this stage however, the basicity of the reagent frequently caused the formation of by-products through the alkaline hydrolysis of the intermediate pthalyl compound. The satisfactory characterisation of these decomposition products was not generally possible, since mixtures were always obtained.

Dr. T. H. Bevan, of these laboratories, has also had difficulty with this reaction in the course of preparing "lyso" cephalins. Several solvents and different temperatures have been tried, but with little success. In the author's experience complete removal of the pthalyl group did not occur unless the substance was refluxed in methoxyethanol with hydrazine hydrate. (These were the conditions used by Rose (loc.cit.) in his original preparation of the cephalins). When the reaction was successful, the yield of product was usually satisfactory, and it seemed that there must be some critical factor which had not been taken into account.

Time did not permit further study, but in the light of the success of this reaction for saturated cephalin, there is little doubt that this method is likely to prove of equal value for the synthesis of unsaturated compounds.

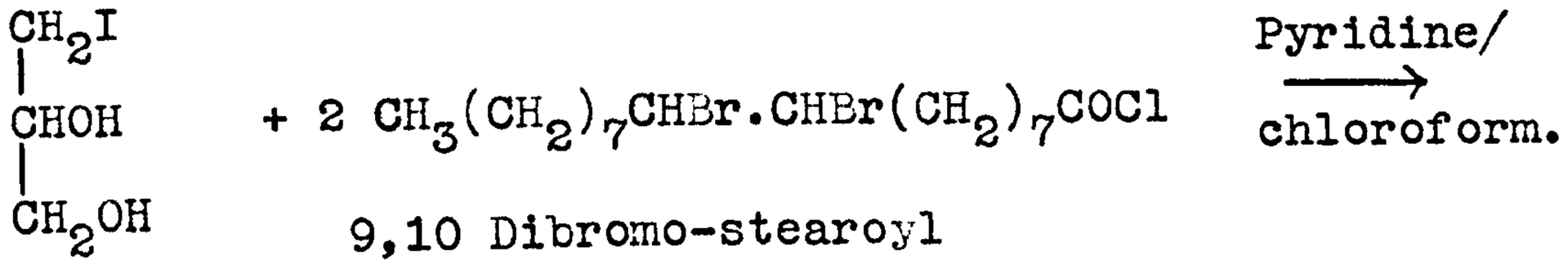
The use of the silver salt of monophenyl N carbobenzoxy ethanolamine phosphate.

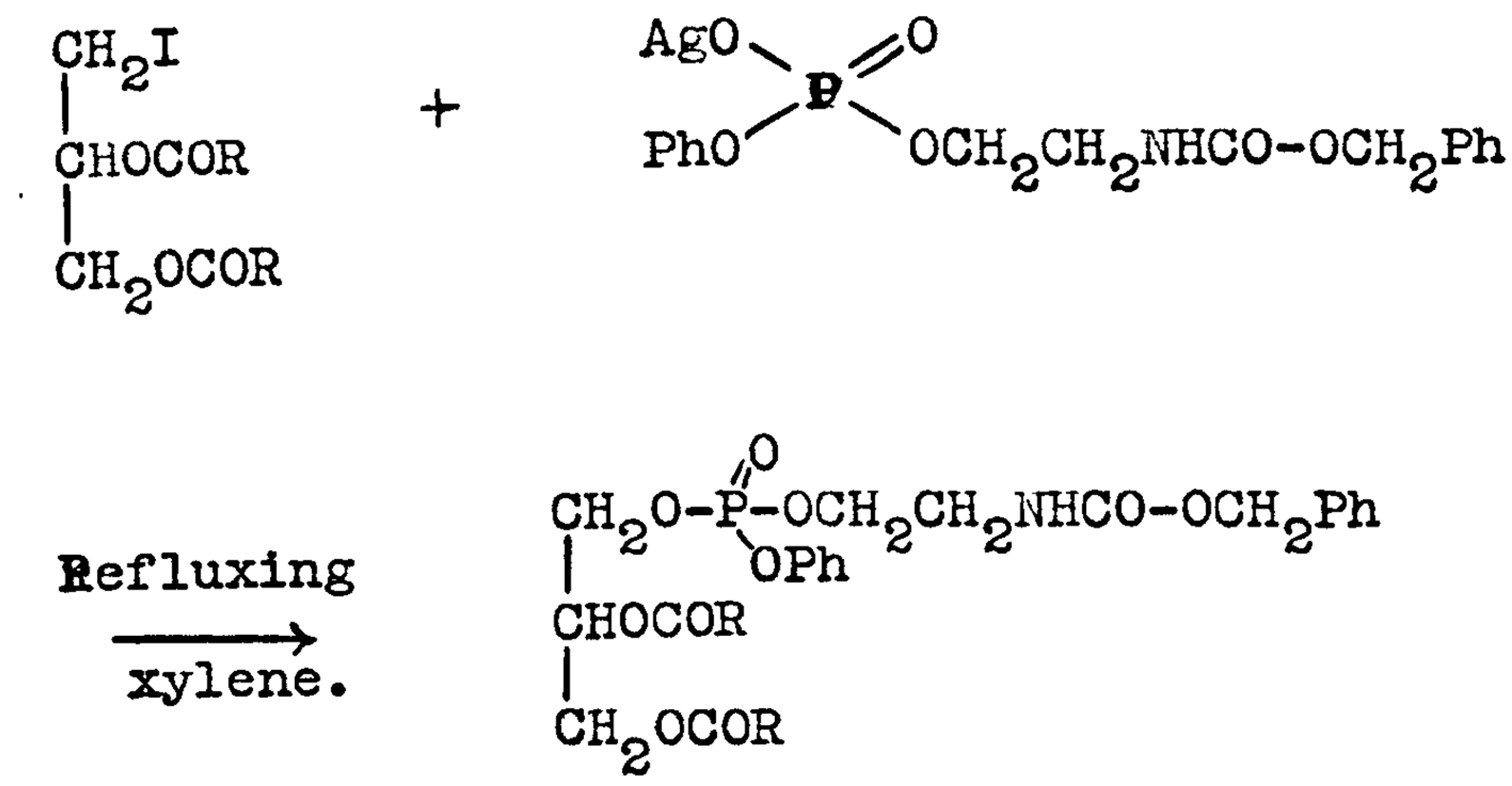
The above silver salt has been used in the preparation of DL saturated cephalins, analogues of cephalin, plasmalogens and L 1,2 diacyl cephalins by various workers in these laboratories. This method was not applicable to the unsaturated compounds owing to the concurrent saturation of the unsaturated acid moieties during the hydrogenolysis of the protecting groups.

If, however, a satisfactory way of protecting the double bonds in the unsaturated acids could be found, this method could then be extended to the unsaturated compounds, and a uniform synthesis of all classes of cephalin phosphatides would have been achieved.

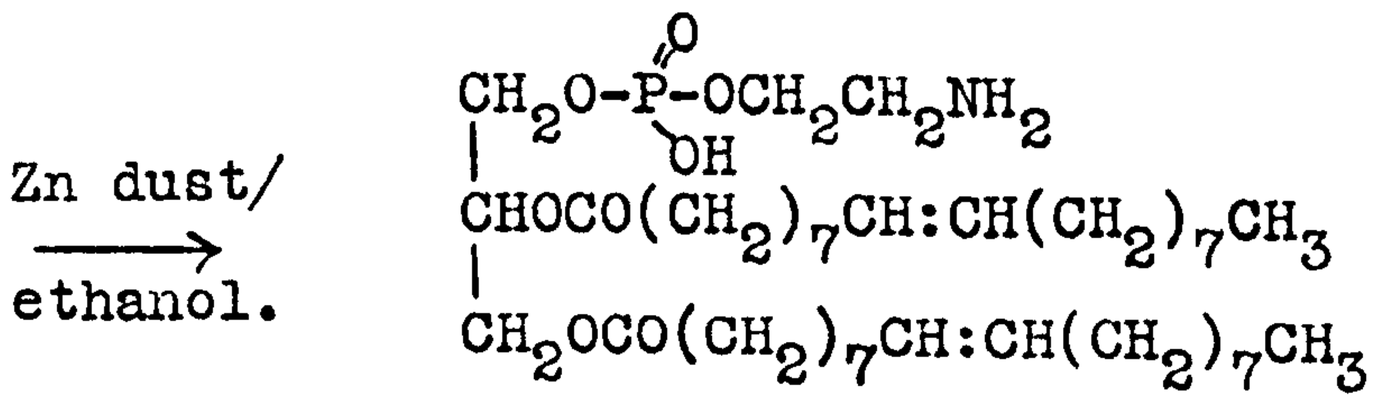
In another part of this dissertation the protection of double bonds by bromination has been described. In view of its success in that instance, it was decided to attempt a synthesis by the route shown on page 125.

Glycerol 1- iodohydrin was acylated with two equivalents of 9,10 dibromostearoyl chloride, and the resulting 1,2 di (9,10 dibromostearoyl) iodohydrin was reacted with the silver salt of monophenyl N carbobenzoxy ethanolamine phosphate in dry xylene, and the product subjected to catalytic hydrogenolysis to remove the protecting groups. The resulting 1,2 di (9,10 dibromo-stearoyl) cephalin was then to be treated with zinc dust in




1,2 Di-(Dibromostearoyl) cephalin.




1,2 Dioleyl cephalin.

ethanol to remove the bromine atoms and yield 1,2 dioleyl cephalin.

This proposed reaction scheme worked satisfactorily until the stage involving catalytic hydrogenation was reached. The dibrominated intermediate would not adsorb hydrogen at room temperature, and it was necessary to resort to elevated temperatures (40-50$^{o}$C ) to effect hydrogenolysis. The product obtained was found to be mainly 1,2 distearoyl cephalin, the bromine protecting atoms being split off as hydrogen bromide. Presumably some form of catalyst poisoning prevented the reaction proceeding at room temperature.

Since this work was completed, Baer has described the preparation of 1,2 di (9,10 dibromostearoyl) cephalin by a direct phosphorylation method similar to that used for saturated compounds. (Baer. "Phosphoric Esters and related compounds" Symposium at Cambridge. April 1957). No experimental details were given but presumably the catalytic hydrogenolysis of the protecting groups presented no difficulties. Baer reports however complete lack of success in the subsequent debromination of this compound to yield the corresponding unsaturated cephalin.

In view of this it appears unlikely that this method will be of use in the preparation of unsaturated phosphatides.

Experimental.

Lecithins.

Direct Phosphorylation Methods.

Throughout the experimental section, if dry solvents and reagents were specified as being used, precautions to exclude moisture were taken by fitting calcium chloride tubes to all outlets and carefully drying all apparatus before use.

Preparation of 2-bromo ethyl phosphoryl dichloride. (Group B.)

62.5 gm. (0.5 mole) of ethylene bromhydrin were added in one portion to 76.75 gm. (0.5 mole) of freshly distilled phosphorus oxychloride at $0^{o}C$, and then the temperature was raised gradually until refluxing occurred. This was continued 30 minutes, and the dark brown liquid was then fractionated at the water pump. After two fractionations 38 gm. (31%) of a clear liquid were obtained b.pt. $105-110^{o}C/15$ m.m.

The anilide was prepared for characterisation - it was a white solid which sintered at $83^{o}C$ and melted at $101-102^{o}C$.

Analysis. Found. N 7.7%

$C_{14}H_{16}O_2PN_2Br$ requires N 7.9%

Phosphorylation of 1,3 distearin with 2-bromo ethyl phosphoryl dichloride.

0.2 gm. (0.0025 mole ) of dry pyridine were added slowly to 0.6 gm. (0.0025 mole) of 2-bromo ethyl phosphoryl dichloride in 5 ml. dry chloroform, and then 1.5 gm. (0.0024 mole) of 1,3 distearin in 50 ml. dry chloroform were added dropwise with stirring over 3 hours. The mixture was stirred overnight at room temperature.

0.1 ml. of water and 0.2 gm. pyridine were added, and stirring was continued a further 6 hours. The solvents were then evaporated under reduced pressure, the "Hi-vac" pump being used to remove last traces. The solid residue was triturated three times with 20 ml. portions of boiling ether, and the ethereal extracts were washed in succession with water, dilute hydrochloric acid, and water (twice). The solution was dried over sodium sulphate, filtered and concentrated under reduced pressure to yield a white solid, which gave an acidic reaction to litmus, and a green flame on copper wire.

Two recrystallisations of a portion of this from 40-60 light petroleum gave a solid, m.p. 75-77$^{o}$C. A mixed m.pt. of this compound with 1,3 distearin was undepressed, and the X-ray side spacings were identical for both compounds. This was conclusive evidence that the product of the reaction was 1,3 distearin and that the phosphorylation had failed.

Phosphorylation of 1,2 distearin with 2-bromo ethyl phosphoryl dichloride.

20 ml. dry pyridine (large excess) were added with cooling to 1.2 gm. (0.0050 mole) of 2-bromo ethyl phosphoryl dichloride, then the mixture was warmed to room temperature and 3.1 gm. (0.0050 mole ) of 1,2 distearin were added portionwise with stirring over 3 hours. Pyridine hydrochloride was precipitated.

After stirring overnight 0.25 ml. of water were added, and stirring was again continued overnight. The solution was now concentrated under reduced pressure, using the "Hi-vac" pump to remove the final traces of pyridine. The resulting solid was triturated with dilute hydrochloric acid, centrifuged, washed free of acid at the centrifuge, and dried in vacuo over concentrated sulphuric acid.

3.4 gm. of crude material were obtained, which after three recrystallisations from 40-60 light petroleum had a m.pt. of 67-9°C. It was acid to litmus and congo red, and gave a green copper flame indicating the presence of halogen. The analysis figures fitted a 50-50 mixture of required product and starting material fairly closely.

| | | | |
|---|---|---|---|
| Analysis. | Found | C 68.5 | H 11.1 |
| Calculated for 50/50 mixture | | C 67.8 | H 11.0 |

of 1,2 distearin and 1,2 distearoyl glyceryl 2-bromo ethyl phosphate.

Preparation of 2-chloroethyl phosphoryl dichloride. (Group B.)

(Plimmer and Burch. Bioch. J. 31 398 (1937)).

39.25 gm. (0.5 mole) of ethylene chlorhydrin were added to ice-cold phosphorus oxychloride, 76.75 gm. (0.5 mole), and then the temperature was gradually raised until the mixture refluxed. After refluxing for one hour the product was distilled at the water pump. B.pt. 103°/12 m.m. of mercury. Yield 61.5 gm. (62%)

Phosphorylation of octadecyl alcohol with 2-chloro-ethyl phosphoryl dichloride.

1.58 gm. (0.02 mole) of dry pyridine were added slowly to a magnetically stirred ice cold mixture of 3.96 gm. (0.02 mole) 2-chloroethyl phosphoryl dichloride and 20 ml. dry chloroform. 5.4 gm. (0.02 mole) octadecyl alcohol dissolved in 60 ml. dry chloroform were now added dropwise with stirring over 3 hours between 0-5°C. Stirring was continued overnight at room temperature.

4 ml. of water and 1.6 gm. pyridine were now added, and stirring was again continued overnight. The mixture was then concentrated under reduced pressure at a bath temperature not exceeding 40°C. The residual white solid was dissolved in ether, washed successively with water, normal hydrochloric acid, and twice more with water. The ethereal solution was then dried

with sodium sulphate.

On evaporating off the ether 7.4 gm. of a waxy white solid m.p. 45-51°C were obtained. After three recrystallisations from 40-60 light petroleum 5.8 gm. (70%) were obtained m.pt. 58-60°C. The pure substance was acid to litmus and gave a positive halogen test.

Analysis. Found C 57.8 H 10.0

$C_{20}H_{42}O_4PCl$ requires C 58.1 H 10.2

Preparation of the sodium salt.

3.0 gm. (0.0073 mole) of octadecyl 2-chloroethyl phosphate were dissolved in ethanol, and the solution was neutralised to phenolpthalein with aqueous sodium carbonate solution. The solvents were evaporated off under reduced pressure below 40°C, and the solid residue was extracted with hot alcohol and the insoluble material (excess sodium carbonate) was filtered. The ethanolic extracts were then concentrated to dryness yielding 3.1 gm. (97%) octadecyl 2-chloroethyl sodium phosphate.

Attempted exchange of the sodium salt of 2-chloroethyl octadecyl phosphate to 2-iodo ethyl octadecyl phosphate.

1.0 gm. (0.0023 mole) of 2-chloroethyl octadecyl phosphate sodium salt were dissolved in 100 ml. calcium sulphate dried methyl ethyl ketone and 1.0 gm. (0.0067 mole; 3 equivalents) of dry sodium iodide were added. The mixture was then refluxed

with stirring for a total of 40 hours.

After this time a small amount of precipitate was filtered off and the filtrate concentrated under reduced pressure to yield a brown solid. This was extracted with dilute sodium hydroxide solution, and the insoluble material was filtered off and recrystallised from ethanol. After two recrystallisations a substance which melted over a range between 35° and 70°C was obtained, but although this contained phosphorus and iodine, the phosphorus analysis figures were approximately one fifth of the theoretical, indicating that degradation had occurred.

Attempted condensation of the sodium salt of 2-chloroethyl octadecyl phosphate with trimethylamine.

1.9 gm. (0.0044 mole) of the sodium salt of 2-chloroethyl octadecyl phosphate were dissolved in dry benzene by warming (about 200 ml. were required) and the solution was transferred to a thick walled reaction tube. 1 gm. (0.017 mole ; 4 equivalents) of anhydrous trimethylamine were weighed out into 30 ml.dry benzene and then added to the tube. The tube was sealed and placed in an oven at 60°C for 4 days. After cooling in liquid air the tube was opened and the contents withdrawn.

The benzene was evaporated under reduced pressure to yield 2.0 gm. of solid residue. Since the appearance and weight of the product were almost identical with the starting material,

a sample was dissolved in water and acidified to precipitate the free acid. After one recrystallisation from 40-60 light petroleum this latter compound had a m.pt. of 54-58°C, and a mixed melting point with an authentic specimen was undepressed. It appeared that no condensation had occurred.

Attempted preparation of monophenyl 2-bromoethyl phosphoryl monochloride. (Group C.)

a) By Direct Refluxing.

6.25 gm. (0.05 mole) of ethylene bromhydrin in 10 ml. dry benzene were added to 11.7 gm. (0.055 mole ; 10% excess) of monophenyl phosphoryl dichloride in 50 ml. refluxing dry benzene, all outlets being guarded by calcium chloride tubes. Refluxing was continued for 5½ hours, then the benzene was distilled off under reduced pressure and the residual pale yellow liquid was distilled in vacuo. Two fractions were obtained:-

1) B.pt. up to 110°C.

2) B.pt. above 110°C - decomposition occurred in the distilling flask.

0.9 gm. of liquid in fraction 1) was obtained. The aniline derivative was prepared for characterisation. After recrystallising from ethanol a white solid m.p. 128°C was obtained.

| | | | | |
|---|---|---|---|---|
| Analysis. | Found. | C 46.9 | H 4.5 | N 4.2 |
| $C_{14}H_{15}O_3NPBr$ | requires | C 47.2 | H 4.2 | N 3.9 |

Although this was the required substance, the yield was too small to be of use.

b) Using quinoline.

6.25 gm. (0.05 mole) of ethylene bromhydrin were added dropwise to a vigonously stirred mixture of 10.6 gm. (0.05 mole) of monophenyl phosphoryl dichloride and 6.45 gm. (0.05 mole) of dry quinoline at a temperature between -15° to -20°C. Quinoline hydrochloride was precipitated. When all the ethylene bromhydrin had been added stirring was continued for a further 30 minutes, then the flask and its contents were allowed to warm to room temperature. During the above operations moisture was excluded by the use of calcium chloride tubes.

The residual pasty mass was extracted three times with 50 ml. portions of dry benzene, and the quinoline hydrochloride was removed by rapid filtration. The filtrates were then concentrated below 40°C to a yellow oil.

Attempted distillation of this oil in high vacuo was unsuccessful. It was decided to prepare an aniline derivative of the residue in an attempt to elucidate its composition. The results were rather unrevealing, no analytically pure compounds being obtained. In the main it appeared that the product was a mixture of monophenyl phosphoryl dichloride and monophenyl 2-bromoethyl phosphoryl monochloride, the former predominating.

Attempted preparation of 1,3-distearoyl lecithin using phosphorus oxychloride as a phosphorylating agent. (Group A.)

To 0.77 gm. (0.005 mole) of phosphorus oxychloride dissolved in 10 ml. dry chloroform and cooled in an ice bath, 2.5 ml. dry pyridine were added dropwise with stirring. A solution of 3.1 gm. (0.005 mole) of 1,3 distearin in 65 ml. dry chloroform/0.25 ml. dry pyridine was added at 10-15°C over one hour, then stirring was continued at 25°C for 30 minutes and at 45°C for 30 minutes.

The temperature was reduced to 10-15°C, and 0.92 gm.(0.005 mole) of choline bromide dissolved in an equal mixture of dry pyridine/NN' dimethylformamide was added over 1 hour. The solution was opalescent at the start of the addition, but cleared later. Stirring at room temperature was continued overnight.

0.5 ml. water were now added and stirring was continued for a further 12 hours. A white solid separated during this process. The solvents were removed under reduced pressure below 40°C, the "Hivac" pump being used to remove last traces. The white solid obtained was triturated with water at the centrifuge, then dissolved in chloroform, washed with dilute hydrochloric acid and water and dried with sodium sulphate.

On evaporation of the chloroform solution 2.5 gm. white solid residue were obtained. This was neutral to litmus, contained no halogen and failed to give a precipitate with an alcoholic solution of cadmium chloride. It was soluble only in

chloroform, and was recrystallised from a mixture of chloroform/ethanol. After three such recrystallisations the m.pt. was 175-6°C.

No precipitate of a reineckate could be obtained with ammonium reineckate solution, and this indicated that the substance was not the choline salt of 1,3 distearoyl phosphatidic acid.

Analysis. Found. C 65.2 H 11.1 N 0.58 P 1.2

These figures corresponded to a substance with the empirical formula $C_{143}H_{292}O_{36}NP$.

Silver Salt Reaction Methods. (Group D.)

Attempted Preparation of Bromo Iodo Ethylene.

Tosylation of Ethylene Bromhydrin.

7.6 gm. (0.04 mole) of p toluene sulphonyl chloride were added portionwise to 5 gm. (0.04 mole) of ethylene bromhydrin in 15 mls. of dry pyridine, the mixture being stirred all the while, and subsequently overnight.

The reaction mixture was drowned out in water, extracted twice with ether, and the ethereal extracts were washed once with water and then dried with anhydrous sodium sulphate.

After evaporation of the ether 5.2 gm. (47%) of a cherry red liquid remained. It was decided not to purify this further

but use it directly for the sodium iodide exchange reaction.

Exchange Reaction with Sodium Iodide in Acetone.

5.2 gm. (0.0185 mole) of 2-O-tosyl ethyl bromide were dissolved in 50 ml. dry acetone and 8.3 gms. (0.055 mole; 3 equivalents) of dry sodium iodide were added. The mixture was refluxed and stirred for 24 hours, and the precipitated sodium p-toluene sulphonate was removed by filtration. After concentration of the filtrates the residue was extracted with ether, washed with sodium thiosulphate solution, and dried with sodium sulphate.

Only a very small quantity of colourless oil (less than 0.5 gm.) was obtained, and this was insufficient for further preparative work.

Cephalins.

Direct Phosphorylation Methods.

Phosphorylation of 1,3 diolein with phosphorus oxychloride, leading to the preparation of 1,3 dioleyl cephalin.

1.4 ml. dry pyridine were added dropwise to an ice cold stirred solution of 0.47 gm. (0.0031 mole) freshly distilled phosphorus oxychloride in 10 ml. dry chloroform. Moisture was excluded from the apparatus with calcium chloride tubes in all outlets. The surrounding water bath temperature was raised

to 10°-15°C, and 1.9 gm. (0.0031 mole) 1,3 diolein (m.p. 27°C) in 20 ml. dry chloroform/0.15 ml. dry pyridine was added dropwise over 1 hour. The mixture was stirred at 25°C for 30 minutes and 45°C for 30 minutes.

After reducing the bath temperature to 10-15°C, 1.17 gm. (0.0062 mole; 2 equivalents) of 2-hydroxy ethyl pthalimide in 45 ml. dry chloroform were added with stirring over 1 hour. Stirring was continued for 30 minutes at 30°C and 30 minutes at 40°C.

The water bath temperature was again reduced to 10-15°C and 0.2 ml. of water were added. The solution was stirred for a further 30 minutes to complete the hydrolysis.

The solvents were evaporated under reduced pressure, final traces of pyridine being removed at the high vacuum pump over 5 hours ($<$40°C). The residual white solid was extracted with three 30 ml. portions of boiling ether, and the ethereal extracts were washed with cold normal hydrochloric acid, twice with water, finally being dried with anhydrous magnesium sulphate.

2.5 gm. (93%) pale yellow oil were obtained on evaporating off the ether. This substance, the acidic intermediate pthalyl compound, was converted immediately to the cephalin.

Conversion to 1,3 dioleyl cephalin.

2.5 gm. (0.0029 mole) of 1,3 dioleyl glyceryl 2-pthalimido ethyl phosphate were dissolved in 25 ml. neutral ethylene glycol monomethyl ether, two drops of bromo-thymol blue indicator were added and the solution was just neutralised with 0.5N potassium hydroxide solution. 0.4 gm. (0.004 mole) of 50% w/v hydrazine hydrate solution were added to the stirred mixture, and the temperature was raised until the solvent refluxed gently. Refluxing was continued for 1½ hours, and then the flask and its contents were cooled to room temperature and kept 2 days in the refrigerator.

The methoxy ethanol mother liquor was decanted off carefully from the viscous oily precipitate, and the latter was dissolved in ether, washed with water to remove traces of hydrazine, and dried with magnesium sulphate.

On evaporating the ether 1.9 gm. (90%) of a waxy solid substance were obtained. This gave a positive ninhydrin test, indicating the presence of an amino group. When a chromatogram was run on Whatman 3MM paper impregnated with silicic acid using an 80/20 mixture of chloroform/methanol as the eluting solvent, the substance gave a band in the solvent front and a spot at Rf = 0.045, while L 1,2 distearoyl cephalin gave a band in the solvent front and a spot at Rf = 0.053.

A sample was recrystallised from ethanol by dissolving it

in the boiling solvent and cooling in the refrigerator for two days. Crystals separated which were centrifuged and dried in vacuo over potassium hydroxide pellets.

The crystalline substance softened on heating at 110°C and gave a meniscus at 173°C.

| | | | | | |
|---|---|---|---|---|---|
| Analysis. | Found | C 65.9 | H 10.4 | N 1.9 | P 4.0 |
| $C_{41}H_{78}O_8NP$ requires | | C 66.3 | H 10.5 | N 1.9 | P 4.2 |

Finally, a 0.3 gm. sample was dissolved in normal hexane and reduced with hydrogen (0.1 gm. Adam's platinum oxide catalyst) to the corresponding 1,3 distearoyl cephalin m.p. 196°C.

| | | | | | |
|---|---|---|---|---|---|
| Analysis. | Found | C 65.5 | H 10.8 | N 1.8 | |
| $C_{41}H_{82}O_8NP$ requires | | C 65.9 | H 11.0 | N 1.9 | |

Preparation of Pure Oleic Acid.

Olive oil was saponified by refluxing for 1 hour with 25% excess alcoholic potassium hydroxide, then the alcoholic solution was evaporated to dryness. The solid residue was dissolved in water, acidified with dilute sulphuric acid, and the water insoluble crude oleic acid was extracted with ether and dried with sodium sulphate.

This crude product was subjected to a process of recrystallisation at low temperatures from acetone. c.f. Brown and

Shinowara JACS. 59 6 (1937). After removing the bulk of the saturated acids present by standing at -20°C for 6 hours, followed by filtration, the filtrate was cooled to -60°C for 1 hour, and the crystalline precipitate of oleic acid was recrystallised a further three times from acetone at -60°C. Finally the almost pure oleic acid was dissolved in a smaller volume of acetone and slowly cooled until crystals started to form (-24°C). These were immediately filtered and the filtrate concentrated to yield pure oleic acid m.pt. 13.0°C.

Preparation of Oleyl chloride.

10 gm. (0.035 mole) of pure oleic acid were heated to 90°C (oil bath) in a flask fitted with a reflux condenser and calcium chloride tube, and 3.5 ml. (5 gm; 0.04 mole) of oxalyl chloride were added. The mixture was heated at 90 - 100°C for two hours, then a further 3.5 ml. of oxalyl chloride were added. After heating for a further 30 minutes, the excess oxalyl chloride was distilled off at the water pump, and the residue was distilled in high vacuum.

Yield 9.0 gm. (87%) b.p. 145-150°C/1 m.m. of mercury.

Preparation of 1,2 diolein.

Preparation of the 1,2 dioleyl 3 benzyl ether of glycerol.

2.7 gm. (0.015 mole) of the 1-O-benzyl ether of glycerol and 2.4 gm. (0.03 mole) dry pyridine were dissolved in 60 ml. dry benzene, and 9 gm. (0.03 mole) of freshly distilled oleyl chloride in 60 ml. dry benzene were added with shaking and cooling. The mixture was then left overnight at room temperature.

300 mls. of ether were added, and the solution was washed successively with N hydrochloric acid, saturated sodium bicarbonate solution, and twice with water, finally being dried with sodium sulphate.

After removal of the ether under reduced pressure, 10.4 gm. (99%) of a yellow oil were obtained.

Bromination in dry ether.

10.4 gm. (0.0147 mole) of 1,2 dioleyl 3 benzyl ether of glycerol were dissolved in 90 ml. dry ether and cooled with stirring to -20°C in an acetone - "Drikold" bath. Bromine was added dropwise until a permanent reddish brown colour was observed. The solution was allowed to reach room temperature, and was then washed with saturated sodium bicarbonate solution (to remove excess bromine) and water. The ethereal solution was dried with anhydrous sodium sulphate.

After evaporation of the solvent under reduced pressure

14.6 gm. (97%) of an oil were obtained. All attempts to crystallise this at temperatures ranging from -20° to - 50°C were unsuccessful. It was decided to hydrogenate the whole of the material and purify it at a later stage.

Hydrogenolysis of the benzyl group.

14.6 gm. (0.0142 mole) of the 1,2 di- (9,10 dibromostearoyl) 3-O-benzyl ether of glycerol were dissolved in 150 ml. of normal hexane, and 2 gm. of palladium black catalyst were added. The mixture was shaken in an atmosphere of hydrogen at a little above atmospheric pressure until uptake ceased. After evacuation of the flask to remove residual hydrogen, the catalyst was filtered and washed with 20 ml. n-hexane.

On concentration of the filtrates 13.2 gm. (99%) of viscous yellow oil were obtained. This was now debrominated without further purification.

Debromination with zinc dust in dry ethanol.

13.2 gm. (0.0141 mole) of 1,2 di-(9,10 dibromostearin) were dissolved in 150 ml. magnesium dried ethanol, and 4.6 gms. acid washed zinc dust were added. The solution was then refluxed for one hour. After cooling to room temperature the zinc dust was filtered through a "Filtercel" pad, the latter well washed with ether, and the combined filtrates were concentrated under reduced pressure. The residual yellow oil was dissolved in ether, washed twice with water, and dried with

magnesium sulphate.

Evaporation of the ether yielded 8.6 gm. of crude product. Recrystallisation of this from n-hexane at -35°C gave 5.7 gm. (66%) of pure 1,2 diolein m.p. 13-14°C.

Analysis. Found. C 75.8 H 11.3

$C_{39}H_{72}O_5$ requires C 75.5 H 11.6

Hydrogenation to 1,2 distearin.

0.5 gm. of 1,2 diolein were dissolved in 50 ml. n-hexane, and 0.25 gm. palladium black catalyst were added. Hydrogenation was commenced and continued until uptake ceased. The catalyst was filtered, washed well with chloroform, and the filtrates were concentrated to dryness under reduced pressure. The white solid obtained was recrystallised from hexane and had a m.pt. 70-72°C alone and mixed with an authentic specimen of 1,2 distearin. Yield 0.45 gm. (90%).

Preparation of 2 pthalyl amino ethyl dichlor-phosphonate.

(Hirt and Berchtold. Helv. Chim. Acta. 40 1928 (1957)).

20 gm. (0.105 mole) of 2-hydroxy ethyl pthalimide and 44 gm. (0.286 mole; 270% excess) phosphorus oxychloride were refluxed in 85 mls. of dry benzene for 4 hours. The benzene and excess phosphorus oxychloride were distilled off under reduced pressure, the "Hivac" pump being used to remove final traces of the latter. The residual viscous oil was dissolved in approximately 50 ml. of

anhydrous ether, cooled in the refrigerator and the flask scratched vigorously to induce crystallisation. The crystalline material was filtered, washed with a little dry ether, and dried in vacuo over potassium hydroxide pellets.

21.8 gm. (67.5%) solid were obtained m.p. 68-71°C.

Attempted Preparation of 1,2 dioleyl cephalin.

2 gm. (0.0032 mole) of 1,2 diolein in 20 ml. dry chloroform were added dropwise to 2 gm. (0.0065 mole;2 equivalents) of 2-pthalyl amino ethyl dichlor-phosphonate and 3 ml. of dry pyridine, all dissolved in 20 ml. dry chloroform. The mixture was stirred magnetically and cooled in a melting ice bath. When the addition was complete (2 hours), stirring was continued at room temperature for two days.

0.2 ml. water and 1 ml. pyridine were added, the mixture was stirred for a further two hours, and then concentrated to dryness under reduced pressure at a bath temperature not exceeding 40°C. Finally the high vacuum pump was used to ensure complete removal of all excess pyridine.

The solid residue was extracted with three 30 ml. portions of boiling ether, and the ethereal extracts were washed with cold N hydrochloric acid, and twice with water. The ethereal solution was then dried with anhydrous magnesium sulphate.

After evaporation of the ether 2.1 gm. (75%) of a viscous

oil were obtained, which was acid to litmus, and gave a positive test for a pthalyl group.

Attempted conversion to 1,2 dioleyl cephalin.

1.7 gm. (0.00195 mole) of 1,2 dioleyl glyceryl 2-pthalimido ethyl phosphate were dissolved in 17 mls ethylene glycol monomethyl ether, neutralised to bromo thymol blue with 0.5N potassium hydroxide solution, then 0.2 gm. (0.002 mole) 50% hydrazine hydrate solution were added. The mixture was stirred at room temperature overnight, then the mother liquor was decanted from the precipitated oil. The latter was dissolved in ether, washed with water and dried with sodium sulphate.

On evaporation of the ether 1.05 gm. (72%) of a yellow oil were obtained. This gave a positive ninhydrin reaction, and when chromatographed on silica impregnated paper gave spots of similar Rf values to a comparison sample of 1,3 dioleyl cephalin. It proved impossible to crystallise this compound from alcohol.

The test for the pthalyl group with resorcinol/sulphuric acid was positive, and in addition the substance was slightly acid. This indicated that the removal of the pthalyl group was incomplete. In an attempt to rectify this 0.4 gm. of the above material were refluxed with 0.5% w/v hydrazine hydrate solution (excess) for 1½ hours, and then cooled in the refrigerator. A white solid m.p. 113°C was obtained, which analysed

approximately for stearoyl hydrazide.

Analysis. Found C 72.4 H 13.8 N 7.6

$C_{17}H_{35}CONH-NH_2$ requires C 72.4 H 12.7 N 9.4

The conditions for this pthalyl removal reaction were very critical, and further work under carefully controlled conditions is required to explain satisfactorily why the method succeeded in some cases and failed in others.

Silver salt reaction methods.

Preparation of 1,2 distearoyl cephalin.

Preparation of Silver Dibenzyl Phosphate.

121 gm. (1.0 mole) of Dimethylaniline and 108 gm. (1.0 mole) Benzyl alcohol were added dropwise over 2 hours with vigorous stirring to an ice cold solution of 68.7 gm. (0.5 mole) phosphorus trichloride in 375 mls.dry benzene. After the addition was complete stirring was continued for 30 minutes, then 54 gm. (0.5 mole ) benzyl alcohol were added over 20 minutes. The mixture was left overnight at room temperature.

250 ml. of water were then added, and the benzene layer was separated. It was successively washed with two 250 ml. portions of water, two 250 ml. portions of 5N ammonium hydroxide solution, and two further 250 ml. portions of water. The benzene solution was then dried over anhydrous sodium sulphate.

The benzene was distilled off under reduced pressure, and benzyl chloride and benzyl alcohol were removed at the high vacuum pump at 100°C and 115°C respectively. The product, crude dibenzyl phospite, weighed 88 gm. (67%).

88 gm. (0.34 mole) of crude dibenzyl phosphite were dissolved in 200 mls. carbon tetrachloride and 80 gms. (83 mls; 1.01 mole) of pyridine and 240 mls. of water were added. The mixture was cooled with stirring to 0°C and 44 gm. (0.275 mole) bromine in 60 ml. carbon tetrachloride were added over 3 hours, the temperature not being allowed to exceed 10°C. After stirring overnight 100 ml. concentrated hydrochloric acid were added, and the carbon tetrachloride layer was separated, washed with water, and neutralised with 10% sodium hydroxide solution. (About 100 mls. were needed). The aqueous layer was separated and acidified to precipitate the oily dibenzyl phosphate. This was extracted three times with chloroform, and the chloroform solution dried with anhydrous sodium sulphate.

The chloroform was evaporated under reduced pressure and the residue was crystallised from a mixture of ether and 40-60 light petroleum. Yield 47.5 gm. (49%) m.p. 79-80°C.

25 gm. (0.09 mole) of dibenzyl phosphate were dissolved in 50 mls. 2N sodium hydroxide solution diluted with 250 mls of water and the solution was neutralised to phenolpthalein with 2N nitric acid. 15.5 gm. (0.09 mole) of silver nitrate in 100mls

water were added dropwise with stirring in the dark, and stirring was continued overnight. The white precipitated silver salt was filtered and dried in vacuo over concentrated sulphuric acid to constant weight. m.p. 211-212°C with decomposition. Yield 28.0 gm. (81%).

This method was first described by

Atherton, Openshaw and Todd, J. 384 (1945).

Atherton, Howard, and Todd. J. 1111 (1948).

Preparation of DL 1,2 distearoyl 3 iodo glycerol.

This was carried out in the same way as described for L 1,2 distearoyl 3 iodo glycerol on Page 71 of Part 1 of this dissertation, except that DL glycerol 1-iodohydrin was used as the starting material instead of the optically active compound.

Reaction of Silver Dibenzyl Phosphate with DL 1,2 distearoyl 3 iodo glycerol.

To a stirred solution of 1.05 gm. (0.00272 mole; 10% excess) of silver dibenzyl phosphate in 50 ml. refluxing dry xylene, 1.8 gm. (0.00245 mole) of DL 1,2 distearoyl glycerol iodohydrin in 15 ml. dry xylene were added, the reaction being carried out under anhydrous conditions in the dark. Refluxing and stirring were continued for 15 minutes, then the solution was allowed to cool and the precipitated silver salts were filtered and washed

with chloroform. The filtrates were concentrated under reduced pressure at a temperature below 40°C.

The residue was dissolved in ether, washed once with saturated sodium bicarbonate solution, then twice with water, and the ethereal solution was dried with anhydrous sodium sulphate.

After filtration of the drying agent and concentration of the solution in vacuo, 2.0 gm. (92%) of white solid residue were obtained. m.pt. 54-6°C after one recrystallisation from ethanol.

Debenzylation with sodium iodide in acetone.

2.0 gm. (0.0023 mole) of 1,2 distearoyl glyceryl dibenzyl phosphate in 80 ml. dry acetone and 0.4 gm. (10% excess) oven dried sodium iodide were refluxed for 15 hours. The flask was then cooled and placed in the refrigerator overnight, and the precipitate was filtered, triturated twice with 10 ml. portions of ether, refiltered and dried in vacuo over potassium hydroxide pellets.

1.5 gm. (81%) of a solid which sintered at 60-70°C and melted at 185°C were obtained.

Preparation of 1,2 distearoyl glyceryl monobenzyl silver phosphate.

To 1.5 gm. (0.0018 mole) of the sodium salt of 1,2 distearoyl glyceryl monobenzyl phosphate in 100 ml. of water, 0.3 gm. (0.0018 mole) of silver nitrate in 5 ml. water were added with stirring in the dark. Stirring was continued for 1½ hours, then the

precipitated silver salt was allowed to settle overnight. After filtering and washing with a little water the solid silver salt was dried in vacuo over concentrated sulphuric acid to constant weight.

1.4 gm. (85%) m.p. 94-6°C.

Tosylation of 2-hydroxyethyl pthalimide.

10 gm. (0.052 mole) of p-toluene sulphonyl chloride were added portionwise with stirring to 10 gm. (0.052 mole) of 2-hydroxyethyl pthalimide in 20 ml. of dry pyridine. Pyridine hydrochloride separated. After the addition was complete (2 hours), the mixture was stirred overnight.

The contents of the flask were then poured into water, and the white solid which separated was filtered, washed well with water, and dried in vacuo over concentrated sulphuric acid. 15.8 gm. crude material m.p. 138-143°C were obtained. After recrystallisation from chloroform/40-60 light petroleum 15.0 gm. (83%) pure 2-tosyl ethyl pthalimide m.p. 143-4°C remained.

Analysis. Found C 58.7 H 4.5 N 3.9

$C_{17}H_{15}O_5NS$ requires C 59.1 H 4.3 N 4.1

Exchange with sodium iodide in acetone to yield 2-iodo ethyl pthalimide.

15.0 gm. (0.043 mole) of 2-tosyl ethyl pthalimide and 19.5gm. (0.130 mole; 3 equivalents) of sodium iodide were refluxed and

stirred together in 450 ml, dry acetone for 24 hours. After cooling, the precipitate of sodium -p-toluene sulphonate was filtered and weighed, (7.8 gm; theoretical for 100% exchange 8.4 gm.) and the acetone filtrate was concentrated to dryness.

The residue was extracted with four 150 ml. portions of ether, and the ethereal extracts were washed with sodium thiosulphate and dried over anhydrous sodium sulphate.

After evaporating the ether under reduced pressure 13.8 gm. of residue remained, which on recrystallising from chloroform/ 40-60 light petroleum yielded 11.4 gm. (88%) of pure 2-iodo ethyl pthalimide m.p. 90-92°C.

| | | | | |
|---|---|---|---|---|
| Analysis. | Found. | C 40.0 | H 2.8 | N 4.4 |
| $C_{10}H_8O_2NI$ requires | | C 39.8 | H 2.66 | N 4.65 |

Reaction of 1,2 distearoyl glyceryl monobenzyl silver phosphate with 2-iodo ethyl pthalimide.

To 1.4 gm. (0.00155 mole) of 1,2 distearoyl glyceryl monobenzyl silver phosphate in 30 ml. refluxing dry xylene, 0.45 gm. (0.00150 mole) 2-iodo ethyl pthalimide in 20 ml. dry xylene were added, and the mixture was refluxed and stirred in the dark for 25 minutes. After cooling to room temperature the precipitated silver iodide was filtered off and washed with chloroform, and the combined filtrates were concentrated under reduced pressure. The residue was dissolved in ether, washed

successively with saturated sodium bicarbonate solution and water, and dried over sodium sulphate.

Evaporation of the ether yielded 1.4 gm. (97%) of a waxy solid, which after two recrystallisations from methanol-ethanol melted at 45-48°C.

Analysis. Found. C 69.5 H 9.4

$C_{56}H_{90}O_{10}NP$ requires C 69.3 H 9.4

Debenzylation with sodium iodide in acetone.

1.2 gm. (0.00124 mole) of 1,2 distearoyl glyceryl monobenzyl 2-pthalyl amino ethyl phosphate and 0.1 gm. (10% excess) dry sodium iodide were refluxed together in 50 ml. dry acetone for 13 hours. A solid separated on cooling, which was filtered, washed with a little cold acetone, and dried in vacuo over potassium hydroxide pellets.

0.95 gm. (87%) of 1,2 distearoyl glyceryl 2-pthalyl amino ethyl sodium phosphate were obtained, which after recrystallisation from ethanol sintered at 73-5°C and melted at 79-80°C.

Analysis. Found. C 65.2 H 9.5 N 1.6

$C_{49}H_{83}O_{10}NPNa$ requires C 65.5 H 9.25 N 1.55

Conversion to DL 1,2 distearoyl cephalin.

0.7 gm. (0.00078 mole) of the sodium salt were dissolved in 40 ml. methoxy ethanol with warming, and after addition of 0.1 gm. (0.001 mole) of 50% w/v hydrazine hydrate solution the mixture

was refluxed with stirring for one hour, then stirred overnight at room temperature.

A precipitate separated on standing at 0°C, which was centrifuged, triturated with three 10 ml. portions of boiling ether and then recrystallised from ethanol.

0.3 gm. (52%) of 1,2 distearoyl cephalin m.pt. 195°C were obtained. Characteristic spherulites were visible through the polarising microscope.

| | | | | |
|---|---|---|---|---|
| Analysis. | Found. | C 65.5 | H 10.7 | N 2.1 |
| $C_{41}H_{82}O_8NP$ requires | | C 65.9 | H 11.0 | N 1.9 |

Attempted preparation of 1,2 dioleyl cephalin.

Preparation of 1,2 dioleyl 3 iodo glycerol.

The details of this preparation corresponded closely with those for the stearoyl compound, except that the product, which was liquid at room temperature, was recrystallised from ether/methanol at -50°C.

Reaction of silver dibenzyl phosphate with 1,2 dioleyl 3 iodo glycerol.

To 2.6 gm. (0.0068 mole) of silver dibenzyl phosphate in 80 ml. refluxing dry xylene, 4.4 gm. (0.006 mole) of 1,2 dioleyl glycerol iodohydrin in 10 ml. dry xylene were added. The mixture was refluxed and stirred in darkness for 25 minutes, then allowed to cool. After precipitated silver iodide had

been filtered, the filtrates were concentrated under reduced pressure.

The residue was dissolved in ether, washed with saturated sodium bicarbonate solution, then with water, and dried over anhydrous sodium sulphate. Evaporation of the ether yielded 5.0 gm. (95%) of an oil.

Debenzylation with sodium iodide in acetone.

5.0 gm. (0.0057 mole) of 1,2 dioleyl glyceryl dibenzyl phosphate were dissolved in 50 ml. dry acetone and 1.0 gm. (0.0061 mole) of dry sodium iodide added. After refluxing for 15 hours the flask was cooled for several hours in the refrigerator, and the acetone was decanted from the viscous precipitate. The latter was washed with a little cold acetone and dried in vacuo over concentrated sulphuric acid.

Yield of 1,2 dioleyl glyceryl monobenzyl sodium phosphate 2.9 gm. (63%).

Precipitation of the silver salt of 1,2 dioleyl glyceryl monobenzyl phosphate.

2.9 gm. (0.0036 mole) of the above sodium salt were dissolved in 30 ml. ethanol, and 0.6 gm. (0.0036 mole) silver nitrate in 5 ml. of water diluted with 10 ml. of ethanol were added. It was important to reduce the amount of water used to the absolute minimum, to prevent co-precipitation of the 1,2 dioleyl glyceryl monobenzyl sodium salt, since this was almost

been filtered, the filtrates were concentrated under reduced pressure.

The residue was dissolved in ether, washed with saturated sodium bicarbonate solution, then with water, and dried over anhydrous sodium sulphate. Evaporation of the ether yielded 5.0 gm. (95%) of an oil.

Debenzylation with sodium iodide in acetone.

5.0 gm. (0.0057 mole) of 1,2 dioleyl glyceryl dibenzyl phosphate were dissolved in 50 ml. dry acetone and 1.0 gm. (0.0061 mole) of dry sodium iodide added. After refluxing for 15 hours the flask was cooled for several hours in the refrigerator, and the acetone was decanted from the viscous precipitate. The latter was washed with a little cold acetone and dried in vacuo over concentrated sulphuric acid.

Yield of 1,2 dioleyl glyceryl monobenzyl sodium phosphate 2.9 gm. (63%).

Precipitation of the silver salt of 1,2 dioleyl glyceryl monobenzyl phosphate.

2.9 gm. (0.0036 mole) of the above sodium salt were dissolved in 30 ml. ethanol, and 0.6 gm. (0.0036 mole) silver nitrate in 5 ml. of water diluted with 10 ml. of ethanol were added. It was important to reduce the amount of water used to the absolute minimum, to prevent co-precipitation of the 1,2 dioleyl glyceryl monobenzyl sodium salt, since this was almost

completely insoluble in water.

The oily precipitate of silver salt was allowed to settle in the dark, then the mother liquor was carefully decanted off, and the oil was dissolved in benzene and dried over magnesium sulphate.

When required for use, the drying agent was filtered, and the solution concentrated under reduced pressure in shaded light.

3.2 gm. (100%) of a viscous oily product were obtained.

Reaction of the silver salt of 1,2 dioleyl glyceryl monobenzyl phosphate with 2-iodo ethyl pthalimide.

This reaction was carried out in a similar manner to the saturated compound. The product was an oil. Yield 2.9 gm. (95%).

Debenzylation of above with sodium iodide and acetone.

2.9 gm. (0.0030 mole) of 1,2 dioleyl glyceryl 2-pthalyl amino ethyl monobenzyl phosphate were dissolved in 60 ml. dry acetone, and 0.5 gm. (0.0033 mole, 10% excess) of dry sodium iodide were added. The solution was refluxed for 20 hours, then cooled in the refrigerator, giving an oily precipitate which was centrifuged, dissolved in ether, transferred to a weighed flask, and the ether evaporated.

1.8 gm. (67%) viscous residue were obtained. The substance gave a positive test for a pthalyl compound.

Attempted conversion to 1,2 dioleyl cephalin.

1.8 gm. (0.0020 mole) of the sodium salt of 1,2 dioleyl glyceryl 2-pthalyl amino ethyl phosphate were dissolved in 80 ml. methoxy ethanol and refluxed with stirring for one hour with 0.2 gm. (0.002 mole) of 50% w/v hydrazine hydrate solution. The mixture was cooled in the refrigerator overnight, when a white solid separated. This was filtered, washed with a little cellosolve and recrystallised twice from ethanol, when the m.pt. was 112-114°C.

The substance was almost insoluble in ether, 40-60 light petroleum and acetone, but soluble in benzene, chloroform, and ethanol. It gave a positive ninhydrin reaction, but did not decolourise a chloroform solution of bromine, indicating lack of unsaturation.

Evidently this was a degradation product produced by alkaline hydrolysis of the intermediate pthalyl compound. No structural assignment could be fitted to analytical results.

The use of the silver salt of monophenyl N carbobenzoxy ethanolamine phosphate.

Preparation of 1,2 di-(dibromostearoyl) 3 iodo glycerol.

3.9 gm. (0.0053 mole) of 1,2 dioleyl 3 iodo glycerol were dissolved in 50 ml. dry chloroform and cooled with stirring to

-10° to -20°C. Liquid bromine was added dropwise with stirring at this temperature until a permanent reddish brown colour appeared. The solution was allowed to warm to room temperature, washed with saturated potassium carbonate solution, water, then dried with anhydrous sodium sulphate.

After evaporation of the ether under reduced pressure 5.6 gm. oily residue remained. It proved impossible to crystallise this even at -50°C. Consequently it was purified by reprecipitation with methanol from its chloroform solution at -45°C, decanting off the mother liquor after the oil had settled.

3.3 gm. (59%) of material were obtained, which was stored in ethereal solution as it tended to decompose in air.

Analysis. Found. C 45.5 H 7.0

$C_{39}H_{71}O_4Br_4I$ requires C 45.7 H 7.2

Reaction between 1,2 di-(9,10 dibromostearoyl)-3 iodo glycerol and the silver salt of monophenyl N carbobenzoxy ethanolamine phosphate.

To 0.9 gm. (0.002 mole) of monophenyl N carbobenzoxy ethanolamine phosphate (silver salt) in 50 ml. refluxing dry xylene, 2.2 gm. (0.002 mole) of the 1,2 di-(9,10 dibromostearoyl) glycerol iodohydrin in 20 ml. dry xylene were added with stirring in the dark. After refluxing for 15 minutes the precipitate of silver iodide was filtered, washed with chloroform, and the filtrates concentrated under reduced pressure at a temperature

not exceeding 40°C.

The residual oil was dissolved in ether, washed once with saturated sodium bicarbonate solution, twice with water, and dried over magnesium sulphate. Yield 2.0 gm. (70%).

Attempted hydrogenolysis of protecting groups.

The oil was dissolved in 100 ml. glacial acetic acid and shaken in an atmosphere of hydrogen at room temperature with 0.7 gm. Adam's platinum oxide and 0.7 gm. Palladium black catalysts. Some 400 mls. of hydrogen were taken up, then uptake ceased. After evacuation of the flask to remove excess hydrogen, filtration of the catalyst and concentration of the filtrates in vacuo, a yellow oily substance was obtained. This gave a positive halogen test (green flame on copper wire), indicating that bromine was present, and a positive ninhydrin reaction, suggesting that the carbobenzoxy group alone had been removed by the hydrogenolysis, and the phenyl group had not been affected.

The product was therefore dissolved in 100 ml. glacial acetic acid in a flask equipped with a magnetic stirrer and hotplate, 2.0 gm. Adam's platinum oxide catalyst were added, and the mixture was vigorously stirred at 40-50°C in an atmosphere of hydrogen. A further 600 ml. were taken up, including that required to saturate the catalyst.

After cooling, filtering the catalyst and washing it with

chloroform, the filtrates were concentrated under reduced pressure to yield a solid residue. This was triturated with three 30 ml. portions of boiling ether, and recrystallised from ethanol.

The substance , a white microcrystalline solid, softened slightly on heating above 150°C, finally giving a meniscus at 190-192°C. Although the halogen test was still faintly positive, analysis figures indicated that the compound was impure 1,2 distearoyl cephalin.

| | | | | |
|---|---|---|---|---|
| Analysis. | Found. | C 61.9 | H 10.0 | N 2.9 |
| 1,2 Di-(dibromostearoyl)cephalin | | C 46.2 | H 7.3 | N 1.3 |
| 1,2 Distearoyl cephalin | | C 65.9 | H 11.0 | N 1.9 |

Evidently cleavage of the dibrominated double bonds occurred under the rather vigorous conditions necessary to effect hydrogen uptake, leading to the formation of saturated cephalins. A small amount of the required 1,2 di (9,10 dibromostearoyl) cephalin was probably produced, but the separation of this from the 1,2 distearoyl cephalin was impracticable.

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