

UNITED STATES PATENT OFFICE

2,447,715

ESTERS OF DIACYL-GLYCEROPHOSPHORIC ACIDS

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13 Claims. (Cl. 260-326)

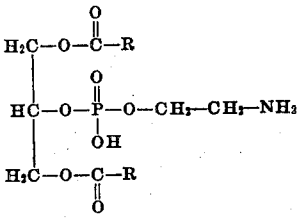
(Granted under the act of March 3, 1883, as amended April 30, 1928; 370 O. G. 757)

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This application is made under the act of March 3, 1883, as amended by the act of April 30, 1928, and the invention herein described, if patented, may be manufactured and used by or for the Government of the United States of America for governmental purposes without the payment to me of any royalty thereon.

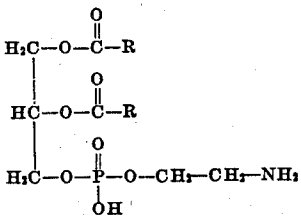
This invention relates to esters of diacyl-glycerophosphoric acids.

More particularly, the invention relates to the preparation of aminoethyl esters of diacyl-glycerophosphoric acids wherein the acyl groups are aliphatic and contain at least eight carbon atoms. These compounds may be illustrated by the following formulae:



(Aminoethyl ester of alpha, gamma-diacyl-glycerophosphoric acid)

and



(Aminoethyl ester of alpha, beta-diacyl-glycerophosphoric acid)

wherein

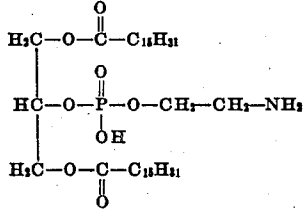


represents an aliphatic acyl radical containing at least 8 carbon atoms.

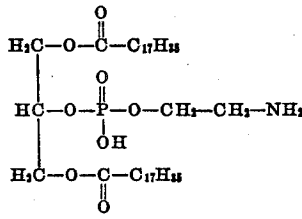
Cephalin is a naturally occurring mixture of aminoethyl esters of diacyl-glycerophosphoric

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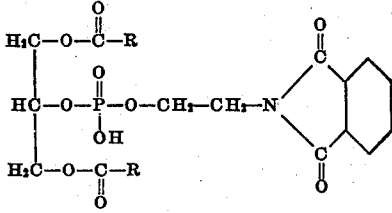
acids. Thus, these esters can also be named with respect to their relationship to cephalin. For instance, the following compound can be named the aminoethyl ester of alpha, gamma-dipalmitoglycerophosphoric acid or alpha, gamma-dipalmitocephalin:



and the following compound can be named the aminoethyl ester of alpha, gamma-distearoglycerophosphoric acid or alpha, gamma-distearocephalin.



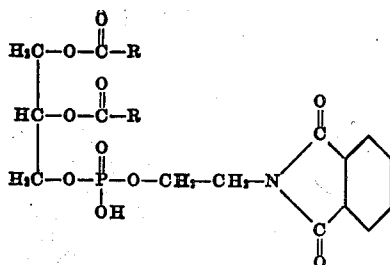
This invention relates with further particularity to the preparation of novel compounds, namely, phthalimidoethyl esters of diacyl-glycerophosphoric acids wherein the acyl groups are aliphatic and contain at least eight carbon atoms. These compounds may be illustrated by the formulae:



(Phthalimidoethyl ester of alpha, gamma-diacyl-glycerophosphoric acid)

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and



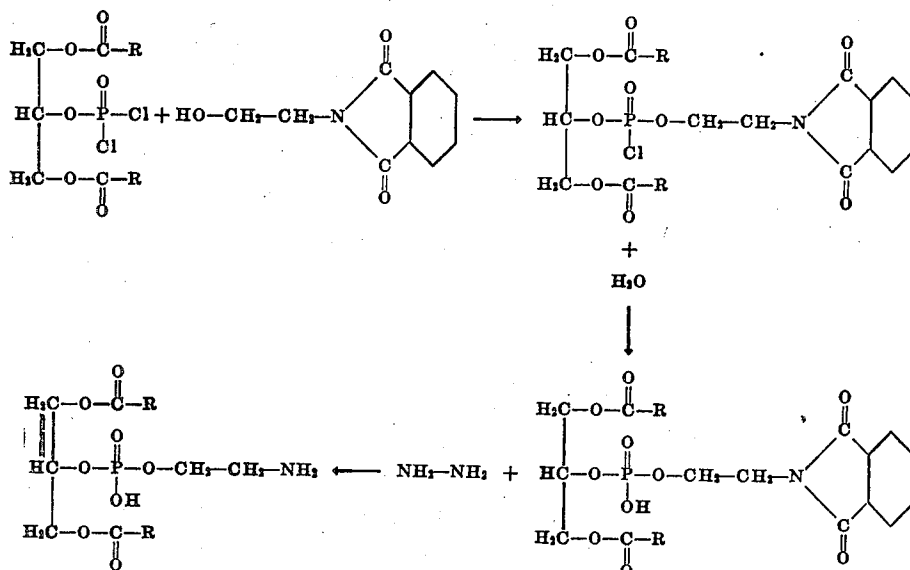
(Phthalimidoethyl ester of alpha, beta-diacyl-glycerophosphoric acid)

wherein



represents an aliphatic acyl radical containing at least 8 carbon atoms.

These novel compounds can also be named



with respect to their relationship to cephalin. Thus, for instance, the phthalimidoethyl ester of alpha, gamma-dipalmitoglycerophosphoric acid can be referred to as alpha, gamma-dipalmitophthalylcephalin.

Accordingly, an object of this invention is to prepare aminoethyl esters of diacyl-glycerophosphoric acids wherein the acyl groups are aliphatic and contain at least eight carbon atoms.

Another object of this invention is to prepare phthalimidoethyl esters of diacyl-glycerophosphoric acids wherein the acyl groups are aliphatic and contain at least eight carbon atoms.

A further object of this invention is to provide novel compounds, namely, phthalimidoethyl esters of diacylglycerophosphoric acids wherein the acyl groups are aliphatic and contain at least eight carbon atoms.

Further objects and advantages will be apparent from the description of the invention.

Methods of preparing the aminoethyl esters are known but are arduous, expensive and give poor yields. One method involves heating distearin with phosphoric anhydride and adding ethanolamine carbonate to this reaction product. This procedure has been found to be unsatisfactory because of reaction between the amine group of the ethanolamine and the diacyl-glycerophosphoric anhydride to form a diacyl-glycerophos-

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phoric acid amide instead of the desired ester. Another method involves the heating together of bromethylamine picrate and the monosilver salt of dipalmitoglycerophosphoric acid. This procedure gives very poor yields probably due to formation of piperazine derivatives.

I have found that the aminoethyl esters can be obtained in good yield by the reaction of a diacylglycerophosphoryl chloride with beta-hydroxyethylphthalimide. The resulting compound, phthalimidoethyl ester of diacyl-glyceromono-chlorophosphoric acid, is subjected to a limited hydrolysis to remove the chlorine atom attached to the phosphorus atom whereby the phthalimidoethyl ester of the diacyl-glycerophosphoric acid is produced. This material is then cleaved by reaction under refluxing conditions with hydrazine, hydrazine hydrate, or the mineral acid salts of hydrazine to produce the aminoethyl ester of the diacyl-glycerophosphoric acid. The reactions can be demonstrated by the following equations:

The following example, which describes the preparation of alpha, gamma-dipalmitophthalylcephalin (A), and of alpha, gamma-dipalmitocephalin (B), discloses particular steps and conditions within the scope of this invention, but it is to be understood that this example is given only by way of illustration and not limitation.

#### 55 (A) Preparation of alpha, gamma-dipalmitophthalylcephalin

Phosphorus oxychloride in the quantity of 9.18 grams was placed in a two-necked flask with 30 ml. of dry pyridine and 40 ml. of chloroform. The flask was provided with an agitator adapted to scrape the bottom of the flask and was surrounded by a water bath at 10°-15° C. The agitator was started and then 34.2 grams of alpha, gamma-dipalmitin in 200 ml. of alcohol-free chloroform was added during the course of 1 hour. The solution was stirred at 25° C. for 30 minutes, then at 30°-35° C. for 30 minutes, then cooled to 10°-15° C. again, and 11.45 grams beta-hydroxyethylphthalimide in 200 ml. chloroform added during the course of 1 hour. The solution was then stirred at 25° C. for 30 minutes and at 30°-35° C. for 30 minutes to complete the reaction. After cooling to 28° C., 1.15 ml. of water in 4 ml. pyridine was added. This step causes the hydrolysis of the chlorine atom. Most of the

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chloroform was then removed with the aid of a water pump vacuum, and ice and water were thereafter added to the residue. The mixture was transferred to a separatory funnel with the aid of ether, and was shaken moderately. The emulsified aqueous layer was withdrawn, acidified, and the ether that separated added to the main ether solution. The ether layer was washed with dilute hydrochloric acid and with water and was then filtered through a thick soft paper or through cotton and left overnight at 5° C. Filtration removed 3.4 grams of material that melted at 50°–60° C., but did not become clear until 160° C. To obtain the principal reaction product, the filtrate was next left for 5 hours at –18° C. and filtered at this temperature. The precipitate retained much ether and was air-dried overnight, as it melted in the retained solvent when it was dried in a vacuum desiccator without preliminary air drying. The dried product weighed 39.5 grams and melted at 43°–46° C. It was found later to be largely phthalylcephalin, but contained several other substances.

The crude material was purified by dissolving it in 600 ml. of hot hexane, filtered hot and allowed to stand 18 hours at room temperature. Filtration gave 4.8 grams of impure phthalylcephalin that sintered at 63° C. and melted from 70°–76° C. This was dissolved in 50 ml. of methanol, filtered hot and allowed to stand at room temperature overnight. Filtration gave 3.0 grams of crystals of phthalylcephalin that sintered at 64° C. and melted at 67°–68° C. The hexane filtrate (above) contained still more phthalylcephalin. It was concentrated to 200 ml., seeded with some of the crystals from the methanol crystallization and thereupon deposited more crystals when allowed to stand at room temperature for 3 hours with occasional stirring. Filtration gave 14.7 grams that sintered at 60° C. and melted at 64°–73° C. This product was dissolved in 150 ml. of hot methanol and the hot solution was filtered through cotton and allowed to stand for 16 hours at room temperature. Filtration gave 12.5 grams that sintered at 69° C. and melted at 70°–72° C. The two products that had been recrystallized from methanol were combined and recrystallized from 200 ml. of hexane, the solution being filtered after standing 1 hour at room temperature. The yield was 13.25 grams of product (alpha, gamma-dipalmitophthalylcephalin or phthalimidoethyl ester of alpha, gamma-dipalmitoglycerophosphoric acid) that melted at 71°–72° C.

Analysis: Calcd. for  $C_{45}H_{76}O_{10}NP$ : N, 1.70; P, 3.77; C, 65.74; H, 9.32; equiv. wt. 822.1. Found: N, 1.66; P, 3.85; C, 66.09; H, 9.47; equiv. wt. 825.

#### (B) Preparation of alpha, gamma-dipalmitocephalin

Four and eleven-hundredths grams of phthalylcephalin prepared as described above was dissolved in 100 ml. of hot neutral monomethyl ether of glycol and neutralized by the addition of 10 ml. of 0.5 N NaOH. Hydrazine hydrate in monomethyl ether of glycol (7.3 ml. of 1.45 M solution) was added, and the solution heated on a steam bath, under reflux for 30 minutes. The solution was cooled, 10 ml. of 6 N hydrochloric acid was added, and the solution was then allowed to stand at room temperature for 15 minutes. The reaction mixture was then poured into 1 liter of cold water and allowed to stand for 2 hours. The product separated out as a finely divided solid.

Removal of the alpha, gamma-dipalmitoceph-

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lin was accomplished by taking advantage of the unexpected property that it is insoluble in ether but is very readily suspended therein. The water suspension was transferred to a separatory funnel with 300 ml. of ether, shaken and the aqueous layer removed. The ether layer and the solid that collected at the ether-water interface were washed with water until the washings were neutral. The ether containing the suspended solid was then filtered, washed with ether and dried. The dry residue amounted to 2.9 grams and melted at 178°–200° C. Recrystallization from 190 ml. absolute alcohol gave 2.47 grams that sintered at 187° C. and melted with decomposition at 192°–193° C., depending on the rate of heating. Further recrystallization from alcohol did not alter the melting point. The product was alpha, gamma-dipalmitocephalin or aminomethyl ester of alpha, gamma-dipalmitoglycerophosphoric acid.

Analysis: Calcd. for alpha, gamma-dipalmitocephalin,  $C_{37}H_{74}O_8NP$ : N, 2.02; P, 4.48; C, 64.22; H, 10.78 percent; equiv. wt. 692.0. Found: N (Dumas), 2.07; Amino N (Van Slyke in acetic acid), 1.86; P, 4.50; C, 63.75; H, 10.52 percent; equiv. wt. (titration in neutral alcohol), 689.

It was necessary to add vanadium pentoxide to the substance, as described for phosphoric acid esters by Wagner-Jauregg and Griesshaber (Berichte Deut. Chem. Gesell., vol. 70, p. 1458 (1937)) to obtain correct carbon analyses. Combustion of the substance alone, or with copper oxide gave carbon values that were 2 to 3 percent low.

As set forth above the first step in the process involves the reaction of beta-hydroxyethylphthalimide with a diacyl-glycerophosphoryl chloride. As the latter reactant, many different compounds can be used wherein the acyl radicals are aliphatic and contain at least eight carbon atoms. The acyl radicals can be attached at the alpha and gamma positions of the glycerine nucleus, in which case the phosphoryl chloride group is at the beta position; or, the acyl groups can be attached at the alpha and beta positions, in which case the phosphoryl chloride group is attached at the gamma position.

These diacyl-glycerophosphoryl chlorides are most conveniently prepared by reacting a diacyl ester of glycerine with phosphorus oxychloride. For example, by reacting alpha, gamma-dipalmitin with phosphorus oxychloride in the presence of pyridine, alpha, gamma-dipalmitoglycerophosphoryl chloride can be prepared. Thus, one can employ as the diacyl-glycerophosphoryl chloride reactant the phosphorus oxychloride reaction product with any of the following diacyl esters of glycerine, i. e., alpha, gamma-dicaprylin; alpha, beta-dicaprylin; alpha, gamma-dipelargonic ester of glycerine; alpha, beta-dipelargonic ester of glycerine; alpha, gamma-dicaprin; alpha, beta-dicaprin; alpha, gamma-diundecylic ester of glycerine; alpha, beta-diundecylic ester of glycerine; alpha, gamma-dilaurin; alpha, beta-dilaurin; alpha, gamma-ditridecylic ester of glycerine; alpha, beta-ditridecylic ester of glycerine; alpha, gamma-dimyristin; alpha, beta-dimyristin; alpha, gamma-dipentadecylic ester of glycerine; alpha, beta-dipentadecylic ester of glycerine; alpha, gamma-dipalmitin; alpha, beta-dipalmitin; alpha, gamma-dimargarin; alpha, beta-dimargarin; alpha, gamma-distearin; alpha, beta-distearin; alpha, gamma-dinondecylic ester of glycerine; alpha, beta-dinondecylic ester of glycerine; alpha, gamma-diarachadin; alpha, beta-

diarachadin; alpha, gamma-diolein; alpha, beta-olein; alpha, gamma-dielaiddin; alpha, beta-dielaiddin; alpha, gamma-dipalmitolein; alpha, beta-dipalmitolein; alpha, gamma-dilinolein; alpha, beta-dilinolein; and so forth.

Further, it is possible to use glycerine esters wherein the two acyl groups are dissimilar, for instance—alpha-stearic, beta-palmitic ester of glycerine; alpha-lauric, gamma-palmitic ester of glycerine; alpha-stearic, gamma-oleic ester of glycerine; alpha-palmitic, gamma-linoleic ester of glycerine; and so forth.

Further, if it is not desired to prepare an individual final compound but mixtures, one can employ mixtures of different diesters of glycerine. For instance, one could employ the mixtures of diglycerides formed by heating glycerine and a catalyst with a triglyceride. Thus, mixtures of diglycerides suitable for use in the process could be prepared by heating glycerine with a catalyst and with coconut oil, palm-kernel oil, olive oil, tallow, suet, cottonseed oil, peanut oil, lard, olive oil, whale oil, sardine oil, corn oil, soybean oil, and so forth. Mixtures of diglycerides can also be prepared by reacting an excess of glycerine, in the presence of an esterification catalyst, with mixtures of homologous fatty acids such as the mixture of fatty acids produced by hydrolysis of any of the fats or oils listed above.

The beta-hydroxyethylphthalimide can be prepared by the heating of ethylene oxide with phthalimide in a sealed tube at 170° C. (Gabriel and Ohle, *Ber. Deut. Chem. Gesell.*, vol. 50, p. 820 (1917)). However, it is preferred to use the following technique:

Twenty-eight grams of ethanolamine was added to 59.2 grams of phthalic anhydride, and after the initial heat of reaction had subsided, the mixture was heated at 150° C. for 30 minutes. It was then allowed to cool to about 90° C. and poured into 800 ml. of water. The crystals so obtained were chilled in ice and filtered, giving 46.7 grams of beta-hydroxyethylphthalimide, *M. P.* 126°–127° C.

Analysis: Calcd. for  $C_{10}H_{13}O_3N$ : C, 62.82; H, 4.74; N, 7.33. Found: C, 62.81; H, 4.65; N, 7.40.

In the reaction of the diacyl glycerine ester with phosphorus oxychloride to prepare the diacyl-glycerophosphoryl chloride, it is necessary to add a hydrogen chloride acceptor to the reaction mixture. For this purpose, pyridine, quinoline, dimethyl aniline or other tertiary amines are suitable. The temperature of the reaction should be kept low. It has been found that temperatures from about 10° to about 35° C. are suitable. Preferably, the reaction should be conducted at the lower temperature, about 10°–15° C., and then heating to about 30°–35° C. for a short time to insure complete reaction. The diacyl-glycerine ester and phosphorus oxychloride should be employed in approximately equimolar proportions. It is preferable to employ a solvent in this reaction. Organic solvents such as chloroform, dichloromethane, benzene and so forth are suitable. Any other inert liquid which will dissolve the diacyl-glycerine ester-phosphorus oxychloride complex and the tertiary amine hydrochloride can be used.

It has been found that the diacyl-glycerophosphoryl chloride need not be isolated from the reaction mixture, but the beta-hydroxyethylphthalimide can be reacted with it in situ. In the reaction of the diacyl-glycerophosphoryl chloride with the beta-hydroxyethylphthalimide, it is necessary to employ a hydrogen chloride ac-

ceptor (quinoline, pyridine, diemethylaniline, or other tertiary amine). It has been found to be convenient to add an excess of the hydrogen chloride acceptor in the first reaction (diacyl-glycerine ester and phosphorus oxychloride) whereby sufficient acceptor will remain in the reaction mixture for the second reaction (diacyl-glycerophosphorylchloride and beta-hydroxyethylphthalimide). In regard to the latter reaction, the same temperature, proportions, and solvents are applicable as to the former reaction.

In the hydrolysis step involving the removal of the chlorine atom from the complex phthalimidoethyl ester of diacylglyceromono-chlorophosphoric acid, the amount of water is not critical. At least an equimolar proportion of water should be added but an excess will not be disadvantageous. The temperature during this step should be kept at about 25° C. or less to prevent hydrolysis of the ester linkages. No solvent is necessary in the hydrolysis, but a solvent such as pyridine can be used if desired to assist in transfer of the water to the mixture.

The expression "limited hydrolysis," as used herein, means a controlled hydrolysis whereby only the chlorine atom on the phosphorus atom is hydrolyzed and the ester linkages are not affected.

In the cleavage step, hydrazine is heated with the phthalylcephalin. One mol of the phthalylcephalin requires 1 to 2 mols of hydrazine. In this cleavage step, the hydrazine can be replaced by hydrazine hydrate, hydrazine hydrochloride, hydrazine sulphate, or other hydrazine mineral acid salts. It is preferable to use a solvent; monomethyl glycol ether, monoethyl glycol ether, ethanol and methanol are suitable. The reactants are preferably refluxed to bring about the reaction and to prevent loss of solvent and/or hydrazine.

The aminoethyl esters produced according to the described process can be used to replace lecithin in a variety of pharmaceutical preparations and cosmetics where they are useful as emulsifying and skin-softening agents. These products are also useful as antiblushing agents in chocolates and as water-binding and dispersing agents in oleomargarine. Because of their surface-active properties, the aminoethyl esters are also useful in preparing oil-in-water emulsions of insecticides and fungicides and in promoting the reaction of hydrophilic materials with hydrophobic materials.

The phthalimidoethyl esters of this invention are useful as a convenient source for the preparation of the corresponding aminoethyl esters. The phthalimidoethyl esters are quite stable and can be kept for long periods contrary to the aminoethyl esters. The former can be readily cleaved with hydrazine when aminoethyl esters are required. The phthalimidoethyl esters are generally useful as intermediates from which to prepare many different glycerophosphoric acid derivatives.

The foregoing example indicates methods of purifying the product and intermediates. These steps can, of course, be omitted if it is not desired to obtain a chemically pure grade of material.

In a copending application, Serial No. 754,256, filed June 12, 1947, now U. S. Patent 2,436,699, I have disclosed a method of preparing aminoethyl esters of diacyl-glycerophosphoric acids involving reaction of carbobenzoxyaminoethanol with a diacyl-glycerophosphoryl chloride, followed by

limited hydrolysis, and cleavage with phosphonium iodide.

Having thus described the invention, what is claimed is:

1. A process comprising reacting a diacyl-glycerophosphoryl chloride, wherein the acyl radicals are aliphatic and contain at least 8 carbon atoms, with beta-hydroxyethylphthalimide in the presence of a tertiary amine at a temperature of about from 10° C. to 35° C., subjecting the resulting phthalimidoethyl ester of diacyl-glyceromono-chlorophosphoric acid to limited hydrolysis by reaction with water at a temperature not exceeding about 25° C. to form the phthalimidoethyl ester of diacyl-glycerophosphoric acid, and refluxing this last-formed ester with a member selected from the group consisting of hydrazine, hydrazine hydrate, and the mineral acid salts of hydrazine to produce an aminoethyl ester of a diacyl-glycerophosphoric acid.

2. A process comprising reacting a diacyl-glycerophosphoryl chloride, wherein the acyl radicals are aliphatic and contain at least 8 carbon atoms, with beta-hydroxyethylphthalimide in the presence of a tertiary amine at a temperature of about from 10° C. to 35° C., subjecting the resulting phthalimidoethyl ester of diacyl-glyceromono-chlorophosphoric acid to limited hydrolysis by reaction with water at a temperature not exceeding about 25° C. to form the phthalimidoethyl ester of diacyl-glycerophosphoric acid, and refluxing this last-formed ester with hydrazine hydrate to produce an aminoethyl ester of a diacyl-glycerophosphoric acid.

3. A process comprising reacting an alpha, gamma-diacylglycerophosphoryl chloride, wherein the acyl radicals are aliphatic and contain at least 8 carbon atoms, with beta-hydroxyethylphthalimide in the presence of a tertiary amine at a temperature of about from 10° C. to 35° C., subjecting the resulting phthalimidoethyl ester of alpha, gamma-diacylglyceromono-chlorophosphoric acid to limited hydrolysis by reaction with water at a temperature not exceeding about 25° C. to form the phthalimidoethyl ester of alpha, gamma-diacylglycerophosphoric acid, and refluxing this last-formed ester with a member selected from the group consisting of hydrazine, hydrazine hydrate, and the mineral acid salts of hydrazine to produce the aminoethyl ester of an alpha, gamma-diacylglycerophosphoric acid.

4. A process comprising reacting alpha, gamma-dipalmitoglycerophosphoryl chloride with beta-hydroxyethylphthalimide in the presence of a tertiary amine at a temperature of about from 10° C. to 35° C., subjecting the resulting phthalimidoethyl ester of alpha, gamma-dipalmitoglyceromono-chlorophosphoric acid to limited hydrolysis by reaction with water at a temperature not exceeding about 25° C. to form the phthalimidoethyl ester of alpha, gamma-dipalmitoglycerophosphoric acid, and refluxing this last-formed ester with a member selected from the group con-

sisting of hydrazine, hydrazine hydrate, and the mineral acid salts of hydrazine to produce the aminoethyl ester of alpha, gamma-dipalmitoglycerophosphoric acid.

5. The process of claim 1 wherein the tertiary amine is pyridine.

6. A process comprising reacting a diacyl-glycerophosphoryl chloride, wherein the acyl radicals are aliphatic and contain at least 8 carbon atoms, with beta-hydroxyethylphthalimide in the presence of a tertiary amine at a temperature of about from 10° C. to 35° C. and subjecting the resulting phthalimidoethyl ester of diacyl-glyceromono-chlorophosphoric acid to limited hydrolysis by reaction with water at a temperature not exceeding about 25° C. to form the phthalimidoethyl ester of diacyl-glycerophosphoric acid.

7. A process comprising reacting an alpha, gamma-diacyl-glycerophosphoryl chloride, wherein the acyl radicals are aliphatic and contain at least 8 carbon atoms, with beta-hydroxyethylphthalimide in the presence of a tertiary amine at a temperature of about from 10° C. to 35° C. and subjecting the resulting phthalimidoethyl ester of alpha, gamma-diacyl-glyceromono-chlorophosphoric acid to limited hydrolysis by reaction with water at a temperature not exceeding about 25° C. to form the phthalimidoethyl ester of alpha, gamma-diacyl-glycerophosphoric acid.

8. A process comprising reacting alpha, gamma-dipalmitoglycerophosphoryl chloride with beta-hydroxyethylphthalimide in the presence of a tertiary amine at a temperature of about from 10° C. to 35° C. and subjecting the resulting phthalimidoethyl ester of alpha, gamma-dipalmitoglyceromono-chlorophosphoric acid to limited hydrolysis by reaction with water at a temperature not exceeding about 25° C. to form the phthalimidoethyl ester of alpha, gamma-dipalmitoglycerophosphoric acid.

9. The process of claim 6 wherein the tertiary amine is pyridine.

10. A phthalimidoethyl ester of a diacyl-glycerophosphoric acid wherein the acyl radicals are aliphatic and contain at least 8 carbon atoms.

11. A phthalimidoethyl ester of an alpha, gamma-diacylglycerophosphoric acid wherein the acyl radicals are aliphatic and contain at least 8 carbon atoms.

12. Phthalimidoethyl ester of dipalmitoglycerophosphoric acid.

13. Phthalimidoethyl ester of alpha, gamma-dipalmitoglycerophosphoric acid.

WILLIAM GORDON ROSE.

#### REFERENCES CITED

The following references are of record in the file of this patent:

Sidgwick, "Organic Chemistry of Nitrogen," new edition, 1937, Oxford University Press (London), pages 14 and 15.