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PHOSPHATIDYLALKANOLAMINE DERIVATIVES

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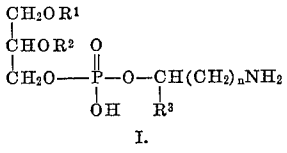
ABSTRACT OF THE DISCLOSURE

There are disclosed herein phosphatidylalkanolamines in which the alkanolamine is 2-hydroxyethyl-, 2- or 3-hydroxypropyl-, or 3-hydroxybutylamine and the acyl groups contain from 18–20 carbon atoms and three or more double bonds. The compounds have anti-hypertensive properties, and methods for their preparation and use are also disclosed.

This invention relates to new phosphatidylalkanolamine derivatives and to processes used for their synthesis.

More specifically, this invention relates to phosphatidylalkanolamine derivatives of Formula I,



I.

in which R^1 and R^2 represent the same or different acyl group containing 18 to 20 carbon atoms and three or more double bonds, such as, for example, an octadeca-6,9,12-trienoyl, octadeca-9,12,15-trienoyl, octadeca-6,9,12,15-tetraenoyl, eicosa-8,11,14-trienoyl or an eicosa-5,8,11,14-tetraenoyl group; n represents the integers one or two; and R^3 represents a hydrogen atom or a lower alkyl group, such as, for example, a methyl group.

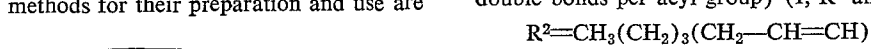
The phosphatidylalkanolamine derivatives of this invention have been found to possess pharmacological properties which render them useful as medicinal agents. More particularly, these derivatives exhibit utility as antihypertensive agents when tested in standard pharmacological tests. For example, when these derivatives are administered to renal hypertensive rats, obtained by the method of A. Grollman, Proc. Soc. Exptl. Biol. Med., 57, 102 (1944), reduction of blood pressure toward normal levels is observed. This fall in blood pressure is readily measured by the method of H. Kersten et al., J. Lab. Clin. Med., 32, 1090 (1947).

When the compounds of this invention are employed as antihypertensive agents in warm-blooded animals, e.g. rats, alone or in combination with pharmacologically acceptable carriers, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard biological practice. For example, they may be administered orally in solid form containing such excipients as starch, milk sugar, certain types of clay and so forth. They may also be administered orally in the form of solutions or they may be injected parenterally. For parenteral administration they may be used in the form of a sterile solution containing other solutes, for example, enough saline or glucose to make the solution isotonic.

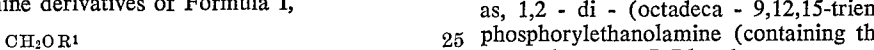
The dosage of the present therapeutic agents will vary with the form of administration and the particular compound chosen. Furthermore, it will vary with the particular host under treatment. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the

circumstances is reached. In general, the compounds of this invention are most desirably administered at a concentration level that will generally afford effective results without causing any harmful or deleterious side effects and preferably at a level that is in a range of from about 0.3 mg. to about 100 mg. per kilo per day, although as aforementioned variations will occur. However, a dosage level that is in the range of from about 3 mg. to about 10 mg. per kilo per day is most satisfactory. Such doses may be administered once or twice a day, as required.

A noteworthy aspect of this invention is the discovery that a minimum of three double bonds must be present in the acyl groups of the phosphatidylalkanolamines of Formula I before said compounds exhibit antihypertensive activity. For example, 1,2-di-(octadeca-9,12-dienoyl)-sn-glycero - 3 - phosphorylethanolamine (containing two double bonds per acyl group) (I; R^1 and

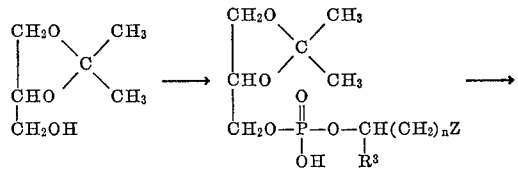


n=1 and R^3=H) when administered by injection at a daily dose of 1 to 10 mg./kg. for five days does not show an antihypertensive effect in renal hypertensive rats according to the method of Kersten et al, cited above; whereas, 1,2 - di - (octadeca - 9,12,15-trienoyl)-sn-glycero-3-phosphorylethanolamine (containing three double bonds per acyl group) [I; R^1 and

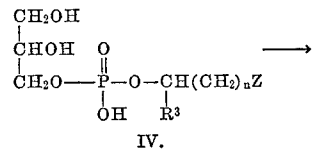


n=1 and R^3=H] exhibits a profound antihypertensive effect at those doese levels.

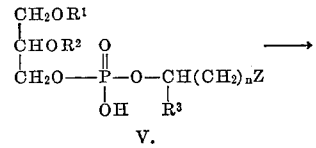
More specifically, I prefer to prepare the phosphatidylalkanolamine derivatives of this invention by the process illustrated by the following formulae:



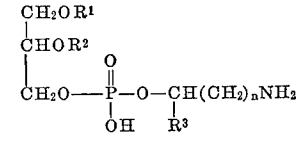
II. III.



IV.



V.



I.

in which R^1, R^2, R^3 and n are as defined above and Z represents a primary amino group coupled with an amine-protecting group, such as, preferably a phthalyl group or some other amine-protecting group, for example, such as those described E. Schröder and K. Lübke in "The Peptides," vol. I, Academic Press, New York, 1965, pp. 3–51.

The starting material of Formula II may be 1,2-isopropylidene-sn-glycerol, described by E. Baer, Biochem. Prep., 2, 31 (1952) or rac-1,2-isopropylidineglycerol, de-

scribed by M. S. Newman and M. Renoll, J. Am. Chem. Soc., 67, 1621 (1945).

The starting material of Formula II is condensed with phosphorus oxychloride in the presence of an organic base, preferably quinoline, followed by treatment with the appropriate 2- or 3-hydroxyalkylphthalimide described below. In this manner the glycerophosphoric acid diester acetonide of Formula III is obtained.

The 2- and 3-hydroxyalkylphthalimides preferred in the above reaction are N-(2-hydroxyethyl)-, N-(3-hydroxypropyl)-, N-(2-hydroxypropyl)- and N-(3-hydroxybutyl) phthalimides. The first two compounds are described by F. Garelli and G. Racciu, Atti accad. sci. Torino, Classe sci. fis., mat. nat., 69, 358 (1934); Chem. Abstr., 29, 6223 (1935); the latter two compounds are described by S. Gabriel and H. Ohle, Chem. Ber., 50, 819 (1917) and R. Robinson and H. Suginome, J. Chem. Soc., 304 (1932), respectively.

The glycerophosphoric acid diester acetonide of Formula III is subjected to mild hydrolyzing conditions, preferably by bringing said diester of Formula III into contact with an acidic ion exchange resin in the presence of an aqueous medium, such as 80% aqueous methanol. Such conditions remove the acetone group and yield the corresponding glycerophosphoric acid diester of Formula IV.

Alternatively, hydrolysis with a dilute acid, such as, for example dilute acetic acid, may be used to achieve the removal of the acetone group yielding the diester of Formula IV.

Before commencing with the next step, the esterification of the free hydroxy groups of the glycerophosphoric acid diester IV, said ester is converted to a corresponding, heavy metal salt, preferably the barium salt. The preparation of this salt serves two purposes. First, a greater stability is imparted to said ester, facilitating its purification and isolation. Secondly, participation of the acidic hydroxy group of the phosphoric acid portion of the molecule in the esterification step is blocked.

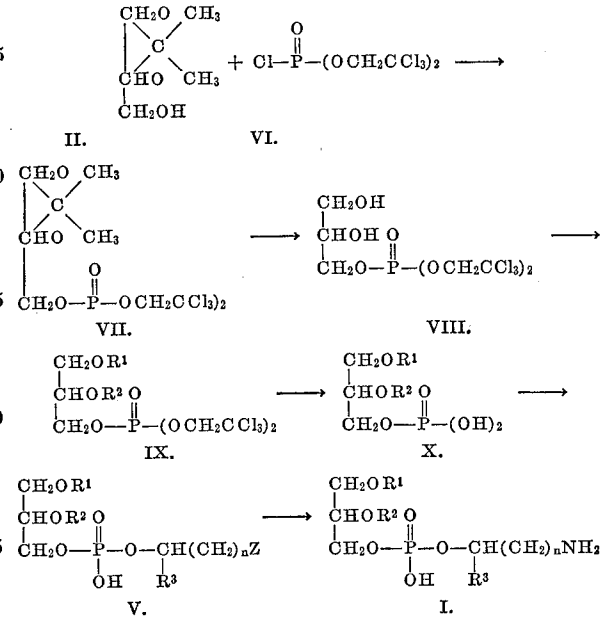
Accordingly, the conversion of the glycerophosphoric acid diester IV to the corresponding diacyl derivative of Formula V is readily achieved by acylation of a heavy metal salt, for example, the barium salt, of the glycerophosphoric acid diester of Formula IV by conventional methods, such as, for example, the use of an acylating agent, such as, an appropriate acid chloride, in the presence of pyridine. When employing the conventional method of using an acid chloride in the presence of pyridine it is an advantage to use dimethylformamide as a solvent for the reaction, since a homogeneous reaction medium, and consequently superior yields are obtained. When this step is used in the process directed to the preparation of the compounds of this invention of general Formula I in which R^1 and R^2 are the same acyl group, an amount more than two equivalents, preferably five equivalents, of the acylating agent are employed. On the other hand, for the process directed toward the compounds of general Formula I in which R^1 and R^2 are different acyl groups, between 0.5 and 1.2 equivalents, preferably 0.8–1.0 equivalent, of the appropriate acylating agent, is first allowed to react with the heavy metal salt of the glycerophosphoric acid diester of Formula IV according to the conventional acylating methods, described above, to yield the corresponding derivative possessing an acylated primary hydroxy group. Subsequent treatment of the latter derivative, also as the heavy metal salt, for example, the barium salt, with an excess, preferably three equivalents of a different acylating agent, chosen accordingly, and using the same acylating conditions, affords the diacyl derivative of Formula V in which R^1 and R^2 represent different acyl groups.

The preferred acylating agents for the above reactions are the corresponding acid chlorides, prepared from the appropriate acids, according to the method

used by W. Stoffel and H. D. Pruss, J. Lipid Res., 8, 196 (1967) for the preparation of octadeca-6,9,12-trienoyl chloride. Such acids are octadeca-6,9,12-trienoic acid, described by J. M. Osbond and J. C. Wickers, Chem. and Ind., 1287 (1959); octadeca-9,12,15-trienoic acid, described by S. S. Nigam and B. C. L. Weedon, Chem. and Ind., 155 (1955); octadeca-6,9,12,15-tetraenoic acid, described by M. Matic, Biochem. J., 68, 692 (1958); eicosa-8,11,14-trienoic acid, described by W. Stoffel, Ann. Chem., 673, 26 (1964); and eicosa-5,8,11,14-tetraenoic acid, described by J. M. Osbond and J. C. Wickers, cited above.

Finally, the protective group for example, the phthalyl group of the diacyl derivatives of Formula V, is removed by conventional treatment with hydrazine hydrate (see for example, E. Schröder and H. Lübke, cited above) to yield the desired phosphatidylalkanolamine derivatives of this invention.

Alternatively, the compounds of this invention may be synthesized by a process illustrated by the following formulae:

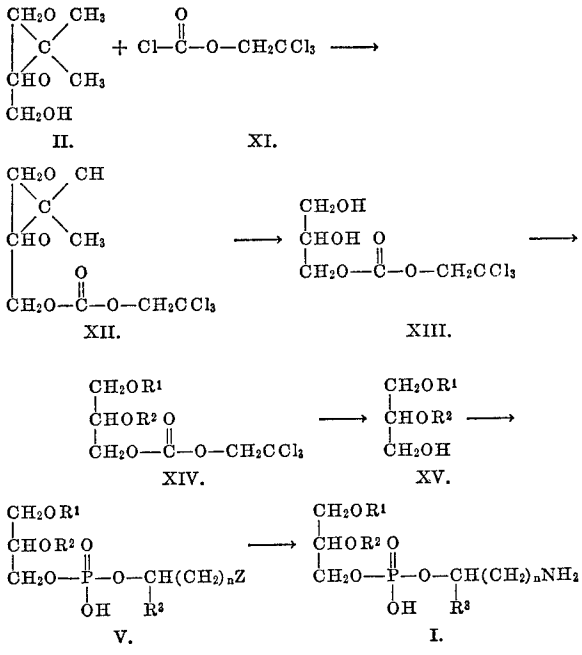


in which R^1, R^2, R^3, n and Z are as defined above.

The starting material of Formula II, described above, is condensed in the presence of an organic base, preferably pyridine, with di-(2,2,2-trichloroethyl)phosphorochloridate (VI) described by F. Eckstein and K. H. Scheit, Angew. Chem. Internat. edit., 6, 362 (1967), to yield the phosphoric acid triester acetonide of Formula VII. The triester acetonide VII is converted to the triester of Formula VIII by mild hydrolyzing methods, such as described above for the conversion of the compounds of Formula III to the compounds of Formula IV. The triester VIII is then treated with appropriate acylating agents, described above, to yield the diacyl phosphoric acid triester of Formula IX. The latter compound on treatment with zinc in acetic acid affords the corresponding diacyl phosphoric acid monoester of Formula X, which may be condensed, for example, in the presence of an organic base, preferably pyridine, and trichloroacetonitrile, with the appropriate 2- or 3-hydroxy-alkylphthalimide, described above, to yield the penultimate intermediate in this process, the phosphatidyl derivative V. This phosphatidyl derviative is also the penultimate intermediate for the preceding process and its conversion to the compounds of this invention of formula is described above.

Again alternatively, the compounds of this invention

may be synthesized by a process illustrated by the following formulae:



in which R^1, R^2, R^3 and n are as defined above and Z represents a primary amino group coupled with a tert-butyloxycarbonyl amine-protecting group.

The starting material of Formula II, described above, is condensed in the presence of an organic base, preferably pyridine with (2,2,2-trichloroethoxy)carbonyl chloride of Formula XI, described by T. B. Windholz and D. B. R. Johnston, Tetrahedron Letters, 2555 (1967), to yield the carbonic acid diester acetonide of Formula XII. The acetone group of the acetonide XII is removed to yield the carbonic acid diester of Formula XIII by mild hydrolyzing methods, such as described above for the conversion of the compounds of Formula III to the compounds of Formula IV. The diester XIII is then treated with appropriate acylating agents, described above, to yield the diacyl carbonic acid diester of Formula XIV. The latter compound, upon treatment with zinc in acetic acid, readily yields the glycerol diacylate of Formula XV which may be condensed with phosphorus oxychloride in the presence of an organic base, preferably quinoline, followed by treatment with an appropriate hydroxyalkylcarbamic acid tert-butyl ester, described below, to afford the compounds of Formula V in which R^1 and R^2 are as defined above and Z represents a primary amino group coupled with a tert-butyloxycarbonyl group.

The appropriate hydroxyalkylcarbamic acid tert-butyl esters preferred in the above reaction are 2-hydroxyethylcarbamic acid tert-butyl ester, described by F. J. M. Daemen et al., Rec. Trav. Chim., 82, 487 (1963), 3-hydroxypropylcarbamic acid tertbutyl ester and 3-hydroxybutylcarbamic acid tert-butyl ester. The latter three esters may be readily prepared according to the procedure of F. J. M. Daemen et al., cited above, used for the preparation of the former ester. When employing this procedure for this purpose, an equivalent amount of 3-amino-1-propanol, described by L. Henry, Chem. Ber., 33, 3169 (1900), 1-amino-2-propanol, described by P. A. Levene and J. Scheidegger, J. Biol. Chem., 60, 172 (1924) and 4-amino-2-butanol, described by R. Robinson and H. Suginome, cited above, is used instead of ethanolamine to obtain the corresponding hydroxyalkylcarbamic acid tert-butyl ester, respectively.

The compounds of Formula V in which R^1 and R^2 are as defined above and Z represents a primary amino group coupled with a tert-butyloxycarbonyl group are readily converted to the phosphatidylalkanolamine derivatives of

Formula I by conventional methods such as those described by E. Schröder and K. Lübke, cited above, see page 39; the use of hydrogen chloride in ether solution is a preferred conventional method.

The following examples will illustrate this invention.

EXAMPLE 1

To a stirred solution of 4.6 ml. of freshly distilled phosphorous oxychloride in 25 ml. of dry methylene chloride at −15° C., is added during 90 min. a solution of 1,2-isopropylidene-sn-glycerol (II, 6.6 g.) and 7 ml. of distilled quinoline in 125 ml. of dry methylene chloride. The reaction mixture is kept at 35° C. for 90 min. The temperature of the reaction mixture is again lowered to 10° C. and over a period of 60 min. a solution of 9.5 g. of N-(2-hydroxyethyl)phthalimide and 16.1 ml. of dry pyridine in 150 ml. dry methylene chloride is added. The reaction mixture is left at room temperature for 18 hours. Water (1.25 ml.) is added to the reaction mixture with continuous stirring and the solvent is removed under reduced pressure at a temperature not exceeding 40° C. The oily residue is triturated successively with three 125 ml. portions of petroleum ether and three 125 ml. of dry ether and then exhaustively extracted with six 125 ml. portions of benzene. The combined benzene extract is evaporated to dryness under reduced pressure at 30–35° C. affording 1,2 - isopropylidene - sn - glycero-3-phosphoryl-N-(2-hydroxyethyl) phthalimide (III; R^3=H, n=1 and Z=phthalimido),

$$\nu_{max.}^{CHCl_3}\ 1775$$

and 1740 cm.$^{-1}$, and a glassy residue. The residue is then dissolved in 250 ml. of 80% methanol and passed through a column of 2.5×40 cm.) Dowex 50 (H^+). The column is rinsed with one liter of 80% methanol. The combined effluents are evaporated under reduced pressure at 35–40° C. to yield sn-glycero-3-phosphoryl-N-(2-hydroxyethyl) phthalimide (IV; R^3=H, n=1 and Z=phthalimido),

$$\nu_{max.}^{CHCl_3}\ 3200\text{–}3350$$

1775 and 1740 cm.$^{-1}$, as a glassy residue. This residue is dissolved in 250 ml. of distilled water and allowed to stand at room temperature for 4 hours, then freed from insoluble materials by extraction with ether. To the clear aqueous solution, 8.75 g. of barium carbonate is added slowly with continuous stirring. After one hour the mixture is filtered and the filtrate centrifuged to remove colloidal particles. The clear supernatant is evaporated under reduced pressure at 35–40° C. to yield a glassy solid. This solid is readily soluble in water, methanol and dimethylformamide and insoluble in ether, benzene and chloroform. It is characterized as the barium salt of sn-glycero-3 - phosphoryl - N - (2-hydroxyethyl)-phthalimide by its analysis: Calculated for $C_{26}H_{30}N_2O_{16}P_2Ba$ (percent): N, 3.39 and P, 7.50. Found (percent): N, 3.40 and P, 7.67.

In the same manner, but using an equivalent amount of N-(3-hydroxypropyl)-, N-(2 - hydroxypropyl)-, or N-(3-hydroxybutyl)phthalimide instead of N-(2-hydroxyethyl)phthalimide, the glycerophosphoryl-N-hydroxyalkylphthalimides, sn - glycero-3-phosphoryl-N-(3-hydroxypropyl)-, -N-(2-hydroxypropyl)-, and -N-(3-hydroxybutyl)-phthalimides and their corresponding barium salts are obtained, respectively.

In the same manner, but using an equivalent amount of rac-1,2-isopropylidineglycerol instead of 1,2-isopropylidene-sn-glycerol, and the appropriate N-(2- or N-(3-hydroxyalkyl)phthalimide, described above, the corresponding racemic mixtures of sn-glycero-3-phosphoryl-N-(2-hydroxyethyl)-, -N-(3-hydroxypropyl)-, -N-(2-hydroxypropyl)-, and -N-(3-hydroxybutyl)phthalimides, and their corresponding barium salts, are obtained.

EXAMPLE 2

To a solution of the thoroughly dried barium salt of the glycerophosphoryl-N-hydroxyalkylphthalimide, sn-

glycero-3-phosphoryl-N-(2-hydroxyethyl)phthalimide IV; R^3=H, n=1 and Z=phthalimido, 2.8 g., prepared as described in Example 1, in 20 ml. of dimethylformamide, is added 5.0 g. of the freshly prepared acyl chloride octadeca-9,12,15-trienoyl chloride, and 2.7 ml. of dry pyridine. The solution is stored under nitrogen at 50° for 48 hours. It is poured into ice and 2 N sulfuric acid. The separated oil is extracted with ether. The ether extract is washed with cold water and dried over sodium sulfate. Removal of solvent gives an oil. The oil is subjected to chromatography using a silicic acid column (40 x 4 cm.). Elution with petroleum ether, benzene mixtures (1:1 and 1:3) and benzene gives compounds showing a negative phosphate test. Elution of the column with 5% methanol in benzene affords the desired phosphatidyl-N-hydroxyalkylphthalimide, 1,2 - di - (octadeca-9,12,15-trienoyl)-sn-glycero-3-phosphoryl-N-(2-hydroxyethyl)phthalimide (V; R^3=H, n=1 and Z=phthalimido),

$$\nu_{max.}^{CHCl_3}\ 3400\text{–}3250$$

1775, 1740 and 1720 cm.$^{-1}$.

In the same manner, but using the acid chlorides, octadeca-6,9,12-trienoyl chloride, octadeca-6,9,12,15-tetraenoyl chloride, eicosa-8,11,14-trienoyl chloride or eicosa-5,8,11,14-tetraenoyl chloride instead of octadeca-9,12,15-trienoyl chloride, the phosphatidyl-N-hydroxyalkylphthalimides,1,2 - di - (octadeca-6,12,15-trienoyl)-, 1,2-di-(octa-6,9,12,15-tetraenoyl)-, di-(eicosa-8,11,14-trienoyl)- and di - (eicosa - 5,8,11,14 - tetraenoyl-sn-glycero-3-phosphoryl)-N-(2-hydroxyethyl)-phthalimides, are obtained, respectively.

In the same manner, but using the acid chlorides, octadeca-6,9,12-trienoyl chloride, octadeca-9,12,15-trienoyl chloride, octadeca-6,9,12,15-tetraenoyl chloride, eicosa-8,11,14-trienoyl chloride or eicosa-5,8,11,14-tetraenoyl chloride and the appropriate glycerophosphoryl-N-hydroxyalkylphthalimides, prepared as described in Example 1, the corresponding, phosphatidyl-N-hydroxyalkylphthalimides, 1,2-di-(octadeca-6,9,12-trienoyl), 1,2 - di-(octadeca - 9,12,15 - trienoyl), 1,2-di-(octadeca-6,9,12,15-tetraenoyl), 1,2-di-(eicosa-8,11,14-trienoyl), 1,2-di-(eicosa-5,8,11,14-tetraenoyl) esters of glycerophosphoryl-N-hydroxyalkylphthalimides, sn-glycero-3-phosphoryl-N-(3-hydroxypropyl)-, -N-(2-hydroxypropyl)-, -N-(3-hydroxybutyl)-phthalimides, are obtained.

In a like manner, the manipulative procedure described in this example may be employed using an appropriate acid chloride, described above, and glycerophosphoryl-N-hydroxyalkylphthalimide, prepared as described in Example 1, in a molar ratio of 0.8:1, followed by a repetition of said manipulative procedure using a three molar excess of a different acid chloride, described above, relative to the amount of said glycerophosphoryl-N-hydroxyalkylphthalimide, to yield the mixed diacylated phosphatidyl-N-hydroxyalkylphthalimides, 1-(octadeca-6,9,12-trienoyl) - 2 - (octadeca-9,12,15-trienoyl)-, -2-(octadeca-6,9,12,15-tetraenoyl)-, -2-(eicosa-8,11,14-trienoyl)-, -2-(eicosa-5,8,11,14-tetraenoyl)-; 1-(octadeca-9,12,15-trienoyl)-2-(octadeca-6,9,12-trienoyl)-, -2-(octadeca - 6,9,12,15-tetraenoyl)-, -2-(eicosa-8,11,14-trienoyl)-, -2-(eicosa-5,8,11,14-tetraenoyl)-; 1 - (octadeca - 6,9,12,15 - tetraenoyl)-2-(octadeca-6,9,12-trienoyl)-, -2-(octadeca - 9,12,15 - trienoyl)-, -2-(eicosa-8,11,14-trienoyl)-, -2-(eicosa-5,8,11,14-tetraenoyl)-; 1-(eicosa-8,11,14-trienoyl)-2-(octadeca-6,9,12-trienoyl)-, -2-(octadeca-9,12,15-trienoyl)-, -2-(octadeca-6,9,12,15-tetraenoyl)-, -2-(eicosa-5,8,11,14 - tetraenoyl)-; 1-(eicosa-5,8,11,14-tetraenoyl)-2-(octadeca-6,9,12-trienoyl)-, -2-(octadeca-9,12,15-trienoyl)-, 2-(octadeca-6,9,12,15-tetraenoyl)-, -2-(eicosa-8,11,14-trienoyl)-sn-glycero-3-phosphoryl-N-(2-hydroxyethyl)-, -N-(3-hydroxypropyl)-, -N-(2-hydroxypropyl)- and -N-(3-hydroxybutyl) phthalimides.

In the same manner, but using the corresponding rac-glycerophosphoryl-N-hydroxyalkylphthalimide, prepared as described in Example 1 instead of the sn-glycerophosphoryl-N-hydroxyalkylphthalimides, employed above in this example, the corresponding rac-phosphatidyl-N-hydroxyalkylphthalimides described in this example, are obtained.

EXAMPLE 3

To a solution of 2.5 g. of the sn-phosphatidyl-N-hydroxyalkylphthalimide, 1,2-di-(octadeca-9,12,15-trienoyl)-sn-glycero - 3 - phosphoryl - N - (2 - hydroxyethyl)phthalimide, prepared as described in Example 2, in 60 ml. of dry ethanol at 0°, 1.25 ml. of a 12.5% hydrazine hydrate solution is added and kept at said temperature for 30 min. Another 1.8 ml. of the same hydrazine solution is added and the mixture is boiled under nitrogen for 2 hours. The solvent is removed under reduced pressure and the residue extracted with ether. The ether extract is mixed with 5 ml. of methanol and 5 ml. of water and the mixture shaken with 2 g. of Dowex 50 (H^+) resin for one hour. The mixture is filtered and the filtrate is concentrated to dryness under reduced pressure. The residue is subjected to chromatography on a column of silicic acid (4 x 30 cm.). The column is exhaustively eluted, first with benzene-chloroform (1:2) and then with chloroform-methanol (9:1). The chloroform-methanol eluates are concentrated to yield the sn-phosphatidylethanolamine, 1,2-di-(octadeca-9,12,15 - trienoyl) - sn-glycero-3-phosphorylethanolamine,

$$\nu_{max.}^{CHCl_3}\ 3500\text{–}3100$$

1735, 1650 and 1245 cm.$^{-1}$.

In the same manner, but using equivalent amounts of the other sn-phosphatidyl-N-hydroxyalkylphthalimides, prepared as described in Example 2, instead of 1,2-di-(octadeca - 9,12,15 - trienoyl) - sn - glycero - 3 - phosphoryl-N-(2-hydroxyethyl phthalamide, the corresponding phosphatidylethanolamines, 1,2 - di - (octadeca-6,9,12 - trienoyl)-, 1,2 - di - (octadeca - 6,9,12,15-tetraenoyl)-, 1,2 - di - (eicosa - 8,11,14 - trienoyl)- and 1,2-di - (eicosa - 5,8,11,14 - tetraenoyl) - sn - glycero - 3-phosphorylethanolamines, are obtained. Similarly, the corresponding sn - phosphatidylethanolamine derivatives, 1,2 - di - (octadeca - 6,9,12 - trienoyl), 1,2 - di - (octadeca - 9,12,15 - trienoyl), 1,2 - di - (octadeca - 6,9,12,15-tetraenoyl), 1,2 - di - (eicosa - 8,11,14 - trienoyl), 1,2-di - (eicosa - 5,8,11,14 - tetraenoyl) esters of sn - glycero-3 - phosphoryl - (3 - hydroxypropylamine), -(2 - hydroxypropylamine), and -(3 - hydroxybutylamine), are obtained. Similarly, the corresponding mixed acylated sn-phosphatidylethanolamine derivatives, 1 - (octadeca-6,9,12 - trienoyl) - 2 - (octadeca - 9,12,15 - trienoyl)-, -2 - (octadeca - 6,9,12,15 - tetraenoyl)-, -2 - (eicosa-8,11,14 - trienoyl)-, -2 - (eicosa - 5,8,11,14 - tetraenoyl)-; 1 - (octadeca - 9,12,15 - trienoyl) - 2 - (octadeca - 6,9,12-trienoyl)-, -2 - (octadeca - 6,9,12,15 - tetraenoyl)-, -2-(eicosa - 8,11,14 - trienoyl)-, -2 - (eicosa - 5,8,11,14-tetraenoyl)-; 1 - (octadeca - 6,9,12,15 - tetraenoyl) - 2-(octadeca - 6,9,12,15 - trienoyl)-, -2 - (octadeca - 9,12,15-trienoyl)-, -2 - (eicosa - 8,11,14 - trienoyl)-, -2 - (eicosa-5,8,11,14 - tetraenoyl)-; 1 - (eicosa - 8,11,14 - trienoyl)-2 - (octadeca - 6,9,12 - trienoyl)-, -2 - (octadeca - 9,12,15-trienoyl)-, -2 - (octadeca - 6,9,12,15 - tetraenoyl)-, -2-eicosa - 5,8,11,14 - tetraenoyl)-; 1 - (eicosa - 5,8,11,14-tetraenoyl) - 2 - (octadeca - 6,9,12 - trienoyl)-, -2 - (octadeca - 9,12,15 - trienoyl)-, 2 - (octadeca - 6,9,12,15-tetraenoyl)-, -2 - (eicosa - 8,11,14 - trienoyl) - sn - glycero-3 - phosphoryl - 2 - ethanolamines, -3 - hydroxypropylamines, -2 - hydroxypropylamines and -3 - hydroxybutylamines, are obtained.

In the same manner, but using the corresponding rac-phosphatidyl-N-hydroxyalkylphthalimides, prepared as described in Example 2 instead of the sn-phosphatidyl-N-hydroxyalkylphthalimides employed above in this example, the corresponding rac-phosphatidylethanolamine derivatives are obtained.

EXAMPLE 4

To a stirred solution of 45.1 g. of di(2,2,2-trichloroethyl)phosphorochloridate (VI) in 300 ml. of dry pyridine at 0° C., 12.6 g. of 1,2 - isopropylidine - sn - glycerol (II) in 50 ml. of pyridine is added dropwise over a period of 30 minutes. The reaction mixture is kept at 0° C. for 12 hours and then concentrated under reduced pressure. The residue is dissolved in chloroform and the resultant solution washed with dilute sodium bicarbonate. Concentration of the chloroform solution yields 1,2-isopropylidine - sn - glycero - 3 - phosphoric acid di - (2,2,2-trichloroethyl)-ester (VII),

$$\delta_{max.}^{CDCl_3}$$ 1.35 (s, 3 protons)

1.45 (s, 3 protons) and 4.75 (s, 4 protons).

In the same manner, but using an equivalent amount of rac - 1,2 - isopropylidene - glycerol instead of 1,2 - isopropylidene - sn - glycerol, rac - 1,2 - isopylidene - glycerol-3 - phosphoric acid di - (2,2,2 - trichloroethyl)ester is obtained.

EXAMPLE 5

A solution of 5.0 g. of 1,2 - isopropylidene - sn - glycero - 3 - phosphoric acid di - (2,2,2 - trichloroethyl)-ester (VII), prepared as described in Example 4, in 250 ml. of 80% methanol is passed through a column of Dowex 50 (H^+). The column is rinsed with one liter of 80% methanol. The combined effluents are evaporated at reduced pressure at 35–40° C., yielding sn - glycero - 3-phosphoric acid di - (2,2,2 - trichloroethyl) - ester (VIII),

$$\nu_{max.}^{CHCl_3}$$ 3600 cm.$^{-1}$ (broad), $$\delta_{max.}^{CDCl_3}$$ 4.75 (s, 4 protons)

In the same manner, but using an equivalent amount of rac - 1,2 - isopropylidene - glycero - 3 - phosphoric acid di - (2,2,2 - tricholroethyl) - ester, prepared as described in Example 4, instead of 1,2 - isopropylidene - sn-glycero - 3 - phosphoric acid di - (2,2,2 - trichloroethyl)-ester, rac - glycero - 3 - phosphroic acid di - (2,2,2 - trichloroethyl)-ester is obtained.

EXAMPLE 6

By using the manipulative procedures and the appropriate acid chlorides, described in Example 2, but substituting an equivalent amount of sn- or rac-glycero-3-phosphroic acid di-(2,2,2-trichloroethyl)-ester, prepared as described in Example 5 in place of the glycerophosphoryl - N - hydroxylalkylphthalimide, followed by treatment with an excess of zinc dust in 80% aqueous acetic acid for one hour at room temperature to remove the 2,2,2-trichloroethyl ester groups the corresponding sn- or rac-phosphatidic acid derivatives, 1,2 - di - (octadeca - 6,9,12-trienoyl)-, 1,2 - di - (octadeca - 9,12,15 - trienoyl)-, 1,2-di - (octadeca - 6,9,12,15 - tetraenoyl)-, 1,2 - di - (eicosa-8,11,14 - trienoyl)-, 1,2 - di - (eicosa - 5,8,11,14 - tetraenoyl)-; 1 - (octadeca - 6,9,12 - trienoyl) - 2 - (octadeca-9,12,15 - trienoyl)-, -2 - (octadeca - 6,9,12,15 - tetraenoyl)-, -2 - (eicosa - 8,11,14 - trienoyl)-, -2 - (eicosa-5,8,11,14 - tetraenoyl)-; 1 - (octadeca - 9,12,15 - trienoyl) - 2 - (octadeca - 6,9,12 - trienoyl)-, -2 - (octadeca-6,9,12,15 - tetraenoyl)-, -2 - (eicosa - 8,11,14 - trienoyl)-, -2 - (eicosa - 5,8,11,14 - tetraenoyl)-; 1 - (octadeca-6,9,12,15 - tetraenoyl) - 2 - (octadeca - 6,9,12 - trienoyl)-, -2 - (octadeca - 9,12,15 - trienoyl)-, -2 - (eicosa - 8,11,14-trienoyl)-, -2 - (eicosa - 5,8,11,14 - tetraenoyl)-; 1-(eicosa-8,11,14 - trienoyl) - 2 - (octadeca - 6,9,12 - trienoyl)-, -2 - (octadeca - 9,12,15 - trienoyl)-, -2 - (octadeca-6,9,12,15 - tetraenoyl)-, -2 - (eicosa - 5,8,11,14 - tetraenoyl)-; 1 - (eicosa - 5,8,11,14 - tetraenoyl) - 2 - (octadeca - 6,9,12 - trienoyl)-, -2 - (octadeca - 9,12,15 - trienoyl)-, 2 - (octadeca - 6,9,12,15 - tetraenoyl)-, -2-(eicosa - 8,11,14 - trienoyl)-, glycero - 3 - phosphoric acids, are obtained.

The 1,2 - di - (octadeca - 9,12,15 - trienoyl) - sn-glycero - 3 - phosphoric acid (X; R^1 and

$$R^2{=}CH_3(CH_2{-}CH{=}CH)_3(CH_2)_7COO)$$

has

$$\nu_{max.}^{CHCl_3}$$ 1750 cm.$^{-1}$ (broad)

EXAMPLE 7

A solution of 7.0 g. of the phosphatidic acid derivative 1,2 - di-(octadeca - 9,12,15 - trienoyl)-sn-glycero-3-phosphoric acid, prepared as described in Example 5, 1.8 g. of the hydroxyalkylphthalimide, N-(2 - hydroxyethyl)phthalimide, and 5.00 g. of trichloroacetonitrile, described by W. Steinkopf, Chem. Ber., 41, 2540 (1908), in 40 ml. of pyridine is heated at 90–100° C. for four hours. The reaction mixture is diluted with water (300 ml.) adjusted to pH 6 with concentrated hydrochloric acid, mixed with ice, and extracted with chloroform. The chloroform extract is dried over sodium sulfate, filtered and evaporated to dryness. The residual oil is subjected to chromatography using a silicic acid column (40× 8 cm.). After elution of the column with petroleum ether, petroleum ether-benzene (1:1) and benzene, elution with 5% methanol in benzene affords 1,2 - di - (octadeca - 9,12,15 - trienoyl)-sn-glycero-3-phosphoric acid,

$$\nu_{max.}^{CHCl_3}$$ 3400–3250

1775, 1740, and 1720 cm.$^{-1}$, identical with the product obtained in Example 2.

In the same manner, but using rac-1,2-di-(octadeca-9,12,15 - trienoyl)-glycero - 3 - phosphoric acid, prepared as described in Example 6, instead of 1,2-di-(octadeca-9,12,15 - trienoyl)-sn-glycero - 3 - phosphoric acid, rac-1,2-di-(octadeca - 9,12,15-trienoyl)-glycero-3-phosphoric acid is obtained.

In the same manner, but using the appropriate sn- or rac-phosphatidic acid derivative, prepared as described in Example 6, and the appropriate 2- or 3-hydroxyalkylphthalimide, described above, the corresponding sn- or rac-phosphatidyl-N - hydroxyalkylphthalimides, described in Example 2, are also obtained.

EXAMPLE 8

A solution of 1.0 g. of (2,2,2-trichloroethoxy)carbonyl chloride (XI) in 5 ml. of dry ether is added slowly to a vigorously stirred ice-cold solution of 500 mg. of 1,2-isopropylidine-sn-glycerol (II) and 1 ml. of pyridine in 5 ml. of dry ether. After stirring at room temperature for one hour, the reaction mixture is diluted with ether. The resultant precipitate of pyridine hydrochloride is removed by filtration. The filtrate is washed successively with cold 2 N-hydrochloric acid and water, dried over sodium sulfate, filtered and evaporated to dryness. The oily product, 1,2 - isopropylidine-sn-glycero - 3 - carbonic acid (2,2,2-trichloroethyl)-ester (XII),

$$\nu_{max.}^{CHCl_3}$$ 1750 cm.$^{-1}$, $$\delta_{max.}^{CDCl_3}$$

1.35 (s, 3 protons), 1.45 (s, 3 protons), 4.75 (s, 2 protons) is not purified further but used for the next step described in Example 9.

In the same manner, but using an equivalent amount of rac - 1,2 - isopropylidine-glycerol instead of 1,2 - isopropylidine-sn-glycerol, rac - 1,2 - isopropylidine-glycero-3-carbonic acid (2,2,2-trichloroethyl)ester is obtained.

EXAMPLE 9

To a solution of 1.1 g. of 1,2 - isopropylidine-sn-glycero-3 - carbonic acid (2,2,2 - trichloroethyl)-ester (XII), prepared as described in Example 8, in 20 ml. of methanol, 0.1 ml. of concentrated hydrochloric acid is added. The resultant solution is kept at room temperature for one hour and then evaporated to dryness under reduced pressure. After drying in high vacuum for 24 hours over sodium hydroxide, the oily residue, sn-glycero-3-carbonic acid (2,2,2 - trichloroethyl)-ester (XIII),

$\nu_{max.}^{CHCl_3}$ 3500 and 1750 cm.$^{-1}$, $\delta_{max.}^{CDCl_3}$ 4.75 (s, 2 protons) is used without further purification for the next step described in Example 10.

In the same manner, but using an equivalent amount of rac - 1,2 - isopropylidine-glycero - 3 - carbonic acid (2,2, 2 - trichloroethyl)-ester instead of 1,2 - isopropylidine-sn-glycero - 3 - carbonic acid (2,2,2 - trichloroethyl)-ester, rac-glycero - 3 - carbonic acid (2,2,2-trichloroethyl)-ester is obtained.

EXAMPLE 10

By using the manipulative procedures and the appropriate acid chlorides, described in Example 2, but substituting an equivalent amount of sn- or rac-glycero - 3 - carbonic acid (2,2,2 - trichloroethyl)-ester, prepared as described in Example 9 in place of the glycerophosphoryl-N-hydroxyalkylphthalimide, followed by treatment with an excess of zinc dust in glacial acetic acid for one hour at room temperature to remove the 2,2,2-trichloroethyl ester group the corresponding sn- or rac-glycerol diacylate derivatives, 1,2 - di-(octadeca-6,9,12 - trienoyl)-, 1,2 - di-(octadeca - 9,12,15 - trienoyl)-, 1,2 - di-(octadeca - 6,9, 12,15 - tetraenoyl)-, 1,2 - di-(eiscosa - 8,11,14 - trienoyl)-, 1,2 - di-(eicosa - 5,8,11,14 - tetraenoyl)-; 1 - (octadeca-6,9,12 - trienoyl) - 2 - (octadeca - 9,12,15 - trienoyl)-, -2-(octadeca - 6,9,12,15 - tetraenoyl)-, -2 - (eicosa - 8,11,14-trienoyl)-, -2-(eicosa-5,8,11,14-tetraenoyl)-; 1-(octadeca-9,12,15 - trienoyl) - 2 - (octadeca - 6,9,12 - trienoyl)-, -2-(octadeca - 6,9,12,15 - tetraenoyl)-, -2-(eicosa - 8,11,14-trienoyl)-, -2-(eicosa - 5,8,11,14 - tetraenoyl)-; 1-(octadeca - 6,9,12,15 - tetraenoyl) -2 - (octadeca - 6,9,12-trienoyl)-, -2-(octadeca - 9,12,15 - trienoyl)-, -2-(eicosa - 8, 11,14 - trienoyl)-, -2-(eicosa - 5,8,11,14-tetraenoyl); 1-(eicosa - 8,11,14 - trienoyl) - 2 - (octadeca - 6,9,10 - trienoyl)-, -2-(octadeca-9,12,15-trienoyl)-, -2-(octadeca-6,9, 12,15-tetraenoyl)-, -2-(eicosa-5,8,11,14 - tetraenoyl)-; 1-(eicosa - 5,8,11,14 - tetranoyl) - 2 - (octadeca - 6,9,12-trienoyl)-, -2-(octadeca-9,12,15-trienoyl)-, 2-(octadeca-6, 9,12,15-tetraenoyl)-, -2-(eicosa - 8,11,14 - trienoyl), glycerols are obtained.

The 1,2 - di-(octadeca - 9,12,15 - trienoyl)-sn-glycerol (XV); R^1 and R^2=$CH_3(CH_2—CH=CH)_3(CH_2)_7COO$ has

$\nu_{max.}^{CHCl_3}$ 3400 and 1745 cm.$^{-1}$

EXAMPLE 11

To a solution of 0.46 g. of freshly distilled phosphorous oxychloride in 10 ml. of dry methylene chloride at 0° C., is added during 30 min. a solution of 1,2 - di-(octadeca-9,12,15 - trienoyl)-sn-glycerol, prepared as described in Example 10, and 0.43 g. of dry quinoline in 10 ml. of dry methylene dichloride. The reaction mixture is kept at room temperature for one hour. The temperature of the reaction mixture is again reduced to 0° C. and a solution of 0.48 g. of 2-hydroxyethylcarbamic acid tert-butyl ester and 0.8 g. of pyridine in 10 ml. of methylene chloride is added over a period of 30 min. After standing at room temperature for 2 hours, 0.05 ml. of water is added and the reaction mixture is stirred for one hour, diluted with 200 ml. of methylene chloride and washed successively with cold 2 N hydrochloric acid and water, dried over sodium sulfate, filters and concentrated under reduced pressure to yield an oil. The oil is purified by chromatography on silicic acid. Elution with 5% methanol in benzene affords the phosphatidyl derivative, 1,2-di-(octadeca-9,12,15-trienoyl)-sn-glycero - 3 - phosphoryl-N-(tert-butyloxycarbonyl)ethanolamine (V); R^1 and

R^2=$CH_3(CH_2—CH=CH)_3(CH_2)_7COO$

R^3=H, n=1 and Z=$NHCOOC(CH_3)_3$,

$\nu_{max.}^{CHCl_3}$

3500–3400, 1745 and 1690 cm.$^{-1}$. This derivative is dissolved in 50 ml. of dry ether and treated with a stream of dry hydrogen chloride at 0° C. for two hours. The solvent is then removed by under reduced pressure at 30° C. The oily residue is subjected to chromatography on 60 g. of silicic acid. Elution with 5–10% methanol in chloroform yields the desired phosphatidylalkanolamine, 1,2-di-(octadeca - 9,12,15 - trienoyl)-sn-glycero - 3 - phosphorylethanolamine,

$\nu_{max.}^{CHCl_3}$

3500–3100, 1735, 1650 and 1245 cm.$^{-1}$, identical with the product obtained in Example 3.

In the same manner, but using the appropriate sn- or rac-glycerol diacylate described in Example 10 instead of 1,2 - di-(octadeca - 9,12,15 - trienoyl)-sn-glycerol together with the appropriate hydroxyalkylcarbamic acid tert-butyl ester, described above, instead of 2-hydroxyethylcarbamic acid tert-butyl ester, the remaining phosphatidylalkanolamines described in Example 3 are obtained.

I claim:

1. 1,2 - di-(octadeca - 9,12,15 - trienoyl)-sn-glycero-3-phosphorylethanolamine.

References Cited

UNITED STATES PATENTS

2,629,662	2/1953	Julian et al.	260—403
2,864,848	12/1958	McArthur	260—403
3,189,628	6/1965	Knight et al.	260—403

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