The bromodeoxycytidine derivative produces a high frequency of plaque-type mutations in T-2 bacteriophage.[9] Screening results at Sloan-Kettering Institute[10] have shown that 5-chlorodeoxycytidine has an inhibitory effect on tumor growth (Ca 755) in rodents, whereas the 5-bromo derivative is inactive.

Discussion

All of the 5-chloro- and 5-bromopyrimidine ribosides and deoxyribosides previously tested competitively inhibit utilization of uridine by the pyrimidine-requiring mutant, Neurospora 1298. The competitive nature of these inhibitions,[5,6] and the fact that the wild-type Neurospora is not inhibited by comparatively high concentrations of these compounds indicate that the primary site of inhibition in the nutant probably involves the conversion of uridine or cytidine to the nucleotide by nucleoside kinase.

In *E. coli*, 15T$^-$ the deoxycytidine derivatives inhibit growth and decrease viability in a manner similar to that described for 5-bromodeoxyuridine.[11] Thus, it is possible that the deoxycytidine derivative exerts its effect by a prior deamination to bromodeoxyuridine and subsequent incorporation into DNA in place of thymidine.[12] However, other interesting changes in DNA structure may result from the presence of the deoxycytidine derivative, such as incorporation into DNA in lieu of deoxycytidine, or formation of an abnormal base as with 5-aminouracil.[13] These possibilities are under investigation.

The high frequency of plaque-type mutations in T-2 bacteriophage produced by bromodeoxycytidine also suggests the possibility that the analog may be incorporated into phage DNA either as the halogenated deoxyuridine, in place of thymidine, if deamination takes place, or as the halogenated deoxycytidine in place of viral 5-hydroxymethyldeoxycytidine. If the latter occurs, it would be an interesting model for chemotherapy application.

(9) J. Gregory, private communication.
(10) Data of D. Clarke, K. Sugiura and C. C. Stock.
(11) S. S. Cohen and H. D. Barner, *J. Bacteriol.*, **71**, 588 (1956).
(12) S. S. Cohen and H. D. Barner, *J. Biol. Chem.*, **226**, 631 (1957).
(13) D. B. Dunn and J. B. Smith, *Nature*, **175**, 336 (1955).

LOS ANGELES, CALIF.

[CONTRIBUTION FROM THE SUBDEPARTMENT OF SYNTHETIC CHEMISTRY IN RELATION TO MEDICAL RESEARCH, BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO]

Synthesis of L-α-(Dioleoyl)-cephalin; with a Comment on the Stereochemical Designation of Glycerolphosphatides[1]

BY ERICH BAER AND DMYTRO BUCHNEA

RECEIVED JUNE 30, 1958

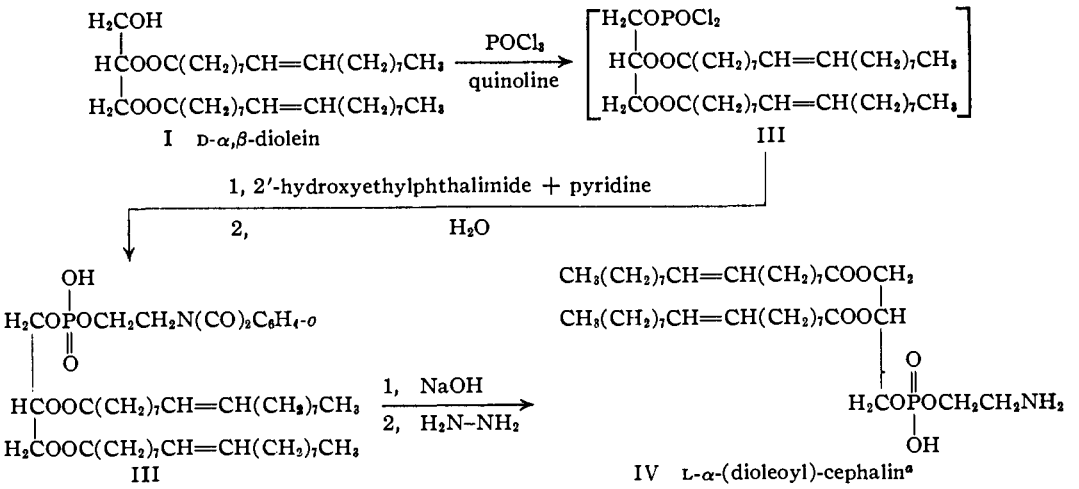
A synthesis of L-α-(dioleoyl)-cephalin is reported. The unsaturated cephalin is obtained by phosphorylation of D-α,β-diolein with phosphorus oxychloride and quinoline, immediate esterification of the resulting L-α-dioleoylglycerylphosphoryl dichloride with 2'-hydroxyethylphthalimide in the presence of pyridine, and removal of the protective phthaloyl group by hydrazinolysis. The configurational and structural purity of L-α-(dioleoyl)-cephalin was confirmed by catalytic reduction to L-α-(distearoyl)-cephalin, and comparison of its specific rotation with that of authentic material. The infrared spectrum of L-α-(dioleoyl)-cephalin, and the solubility of the substance in various solvents are reported.

The synthesis of structurally and configurationally pure glycerolphosphatides containing unsaturated fatty acid residues has lagged behind that of the saturated analogs, although unsaturated phosphatides are known to occur more widely in nature. It is only in the last few years that improvements in the techniques for the separation of lipid mixtures have made possible the synthesis of such unsaturated compounds *via* less protected intermediates than those used in the saturated series. Two years ago we reported the synthesis of L-α-(dioleoyl)-lecithin.[2] The syntheses of the nitrogen-free phosphatides, dioleoyl L-α-glycerylphosphoric acid,[3a] tetraoleoylbis-(L-α-glyceryl)-phosphoric acid[3a] and (dioleoyl-L-α-glycerylphosphoryl)-L-α-glycerol[3b] are reported by us in two recent papers. The purpose of the present paper is to describe the synthesis of L-α-(dioleoyl)-cephalin. It is interesting to note that oleic acid appears to be the principal unsaturated fatty acid in naturally occurring cephalins.[4,5]

As mentioned in an earlier publication[2] the procedures developed in this Laboratory for the synthesis of saturated α-phosphatides, employing phenylphosphoryl dichloride as phosphorylating agent, are not suitable for the synthesis of unsaturated phosphatides, since the removal of the phenyl group by catalytic hydrogenolysis would lead to a simultaneous reduction of the fatty acid double bonds. At the time it was, however, felt desirable to retain the use of phenylphosphoryl dichloride for the synthesis of dioleoyllecithin and a method was devised which made this possible.[2] The L-α-(dioleoyl)-lecithin was obtained *via* the following sequence of intermediates: D-acetoneglycerol → acetone-L-α-glycerylphenylphosphoryl chloride → acetone-L-α-glycerylphenylphosphoryl ethylene chlorohydrin → L-α-glycerylphosphoryl ethylene chlorohydrin → L-α-dioleoylglycerylphosphoryl ethylene chlorohydrin → L-α-dioleoylglycerylphosphoryl-choline. It was hoped that L-α-(dioleoyl)-cephalin could be obtained by using the same procedure,

(1) The synthesis of L-α-(dioleoyl)-cephalin was reported in a lecture presented at a "Symposium on Phosphoric Esters and Related Compounds" held by the Chemical Society at Cambridge, England, April 9-12, 1957.
(2) E. Baer, D. Buchnea and A. G. Newcombe, THIS JOURNAL, **78**, 232 (1956).
(3) (a) E. Baer and D. Buchnea, *Arch. Biochem. and Biophys.*, **78**, 294 (1958); (b) *J. Biol. Chem.*, **232**, 895 (1958).
(4) J. Parnas, *Biochem. Z.*, **22**, 411 (1909).
(5) C. G. MacArthur and L. V. Burton, THIS JOURNAL, **38**, 1375 (1916).

REACTION SCHEME



[a] D- and DL-α-(dioleoyl)-cephalin can be obtained by the same procedure, using as starting materials L- an dDL-α,β-diolein, respectively.

but substituting ammonia for trimethylamine in the final step of the synthesis. We were naturally aware of the possibility that ammonolysis of the fatty acid residues might occur under these conditions. This indeed was found to be the predominant reaction. Changing the reaction medium from benzene to ether, dimethylformamide or pyridine and varying the reaction temperature from 40 to −30° did not prevent this ammonolysis.

On failing to obtain the L-α-(dioleoyl)-cephalin by this method, we investigated the possibility of preparing it by debromination of L-α-bis-(9,10-dibromostearoyl)-cephalin with activated zinc. The brominated cephalin was obtained *via* the following intermediates: D-α,β-(bis-9,10-dibromo)-distearin → L-α-bis-(9,10-dibromostearoyl)-glycerylphenylphosphoryl chloride → L-α-bis-(9,10-dibromostearoyl) - glycerylphenylphosphoryl - N - carbobenzoxyethanolamine → L-α-bis-(9,10-dibromostearoyl)-glycerylphosphorylethanolamine.[6] The brominated cephalin, however, proved to be completely inert to activated zinc under a variety of experimental conditions.

In the meantime the D- and L-α,β-dioleins had become available by debromination of D- and L-α,β-(bis-9,10-dibromo)-distearin, respectively, with activated zinc.[7] This offered the opportunity of preparing L- and D-α-(dioleoyl)-cephalin by a procedure developed by Rose[8] for the synthesis of saturated β-cephalins, and later successfully applied also to the synthesis of saturated α-cephalins by Bevan and Malkin,[9] and of a β-cephalin containing an unsaturated fatty acid substituent by Hunter, Roberts and Kester.[10] A modification of the procedure has recently been developed by Hirt and Berchtold.[11] The original objection to the use of phosphorus oxychloride as phosphorylating agent, that is, the formation of complex mixtures of phosphorylation products that are difficult to separate, has now been overcome by the introduction of the silicic acid column technique for the separation of phosphatides. The unsaturated cephalin was finally obtained by the following procedure. D-α,β-Diolein (I) was phosphorylated with phosphorus oxychloride and quinoline (see Reaction Scheme), and the resulting dioleoyl L-α-glycerylphosphoryl dichloride was treated directly with 2'-hydroxyethylphthalimide and pyridine. On separation of the reaction product on a silicic acid column, pure dioleoyl L-glycerylphosphoryl - 2' - hydroxyethylphthalimide (III) was obtained in a yield of 84% of theory calculated for D-α,β-diolein. Treatment of the N-phthaloylcephalin in the form of its sodium salt with hydrazine at 60°[12] to remove the phthaloyl group gave in good yield a product that analyzed correctly for pure L-α-(dioleoyl)-cephalin. Its chromatographic analysis on paper treated with silicic acid, by the method of Marinetti and Stotz[13] revealed, however, that it contained at least three other compounds. Attempts to remove these impurities by precipitation of the crude material from various solvents by the addition of anhydrous acetone, or by extraction with selective solvents were unsuccessful. The L-α-(dioleoyl)-cephalin was finally obtained in a chromatographically as well as

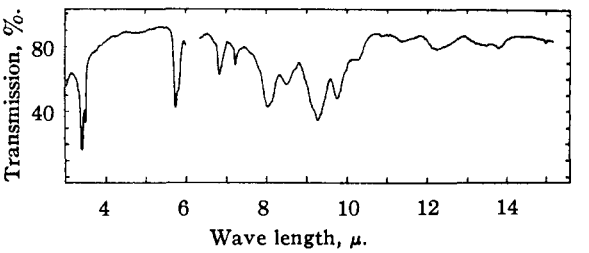


Fig. 1.—Infrared spectrum of L-α-(dioleoyl)-cephalin. A Perkin-Elmer (model 21) double-beam spectrophotometer (sodium chloride prism) was used, KBr disk; sample concentration: 0.6 mg. of cephalin per 1.0 g. of KBr.

(6) Unpublished work.
(7) E. Baer and D. Buchnea, *J. Biol. Chem.*, **230**, 447 (1958).
(8) W. G. Rose, THIS JOURNAL, **69**, 1384 (1947).
(9) T. H. Bevan and T. Malkin, *J. Chem. Soc.*, 2667 (1951).
(10) I. R. Hunter, R. L. Roberts and E. B. Kester, THIS JOURNAL, **70**, 3244 (1948).
(11) R. Hirt and R. Berchtold, *Helv. Chim. Acta*, **40**, 1928 (1957).
(12) This reaction temperature, although much lower than that used by previous workers (8, 9, 10) was found to be high enough for an efficient removal of the phthaloyl group.
(13) G. V. Marinetti and E. Stotz, *Biochim. et Biophys. Acta*, **21**, 168 (1956).

an analytically pure state by a chromatographic separation of the crude product of hydrazinolysis on a silicic acid column.[14] The pure L-α-(dioleoyl)-cephalin (IV), an almost white, waxy material, was obtained from diolein in an over-all yield of 40%. On saponification, it yielded 95% of the theoretical amount of pure oleic acid, indicating that no elaidinization has occurred. Like L-α-(dioleoyl)-lecithin[1] and dipalmitoleoyllecithin[15] it remained colorless on exposure to air. The unsaturated cephalin was found to be readily soluble in chloroform, ether, petroleum ether, methanol and ethanol, but insoluble in anhydrous acetone. Its specific rotation, $[\alpha]_D$ +6.0° in chloroform, was identical with that of L-α-(distearoyl)-cephalin.[16,17] Further proof of the optical and structural purity of L-α-(dioleoyl)-cephalin was obtained by its catalytic reduction in glacial acetic acid with hydrogen and platinum black, which gave in excellent yield L-α-(distearoyl)-cephalin with the specific rotation and melting point of authentic L-α-(distearoyl)-cephalin.[16,17]

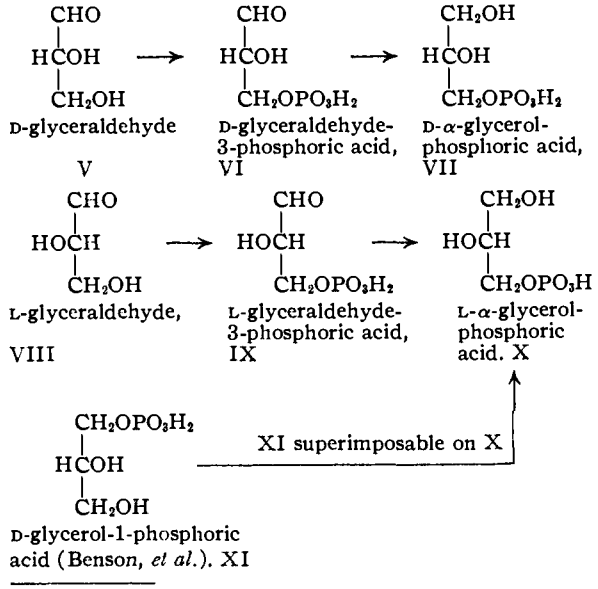
The L-α-(dioleoyl)-cephalin has been tested by Kuhn and Klesse[18] as a substitute for the lipid component of thrombokinase in blood coagulation, and was found to be almost as active as the natural material.

The synthesis of other phosphatides from α,β-diolein is being continued.

The Stereochemical Designation of Glycerolphosphatides.—The synthesis of enantiomeric α-glycerolphosphatides and the concomitant elucidation of the structure and configuration of naturally occurring phosphatides had made it necessary to establish a system for their steric classification, preferably one that would also include the related fatty acid-free glycerolphosphate esters of choline, ethanolamine and serine. Since glycerophosphoric acid is the only moiety common to all, it was made the basis for the steric classification of the glycerolphosphatides.[19,20] The alternate possibility of making the diglycerides the stereochemical compounds of reference for the phosphatides would force one to use two stereochemical systems of reference for glycerolphosphate derivatives, one for the phosphatides and another for the related fatty acid-free biological intermediates, which would have opposite configurational symbols (D and L).

The α-(dioleoyl)-cephalin obtained by phosphorylation of D-α,β-diolein has the configuration represented by the Fischer projection formula IV (Reaction Scheme). Since the phosphoric acid is introduced at the hydroxyl group which was formed by reduction of the carbonyl group of acetone D-glyceraldehyde, it is in a position opposite to that of the phosphoric acid of D-α-glycerolphosphoric acid, the reduction product of D-glyceraldehyde-3-phosphoric acid, and the cephalin therefore is assigned to the L-series.

In a recent paper Benson and Maruo[21] state that L-α-glycerophosphoric acid = D-glycerol-1-phosphate. As in the chemistry of substituted glycerols the prefixes α and 1 have been used interchangeably for many years signifying a terminal substitution of the glycerol molecule without differentiating between the two possibilities,[22] Benson and Maruo's statement, without any qualification as to the precise meaning of 1, appears ambiguous and apt to create confusion. If one adheres to the universally accepted convention for depicting the spatial arrangements of D- and L-glyceraldehyde as shown by projection formulas V and VIII,[23,24] which also express their absolute configurations[25–29], it follows that the spatial arrangements of D-glyceraldehyde-3-phosphoric acid and L-glyceraldehyde-3-phosphoric acid, and the directly related D-α-glycerolphosphoric acid and L-α-glycerolphosphoric acid are expressed by projection formulas VI, IX, VII and X, respectively. By synthesizing both D-α-glycerolphosphoric acid[30] and L-α-glycerolphosphoric acid[31] by a series of reactions which clearly establish their stereochemical relationships to D- and L-glyceraldehyde, the stereochemical compounds of reference, it has been shown that the *levorotatory* D-α-glycerolphosphoric acid and the naturally occurring *dextrorotatory* L-α-glycerolphosphoric acid have the spatial arrangements expressed by formulas VII and X, respec-



(14) In view of the fact that the product of hydrazinolysis had to be purified by chromatography on silicic acid to give pure L-α-(dioleoyl)-cephalin, it might be worthwhile to check the chromatographic purity of previous cephalin preparations obtained by similar routes but purified by recrystallization only.

(15) D. J. Hanahan and M. E. Jayko, THIS JOURNAL, **74**, 5070 (1952).

(16) E. Baer, J. Maurukas and M. Russell, *ibid.*, **74**, 152 (1952).

(17) E. Baer, *Can. J. Biochem. and Physiol.*, **35**, 239 (1957).

(18) R. Kuhn and P. Klesse, *Naturwissenschaften*, **44**, 352 (1957).

(19) H. O. L. Fischer and E. Baer, *Chem. Revs.*, **29**, 287 (1941).

(20) E. Baer and M. Kates, THIS JOURNAL, **72**, 942 (1950).

(21) A. A. Benson and B. Maruo, *Biochim. et Biophys. Acta*, **27**, 189 (1958).

(22) See, for instance, A. W. Ralston, in "Fatty Acids and their Derivatives," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 530.

(23) E. Fischer, *Ber. chem. Ges.*, **24**, 1836, 2683 (1891).

(24) M. A. Rosanoff, THIS JOURNAL, **28**, 114 (1906).

(25) W. Kuhn, *Z. physik. Chem.*, **B31**, 23 (1935).

(26) W. Kuhn, *Z. Elektrochem.*, **56**, 506 (1952).

(27) J. M. Bijvoet, A. F. Peerdeman and A. J. van Bommel, *Nature*, **168**, 271 (1951).

(28) J. M. Bijvoet, *Endeavour*, **14**, 71 (1955).

(29) J. A. Mills and W. Klyne, in "Progress in Stereochemistry," Academic Press, Inc., New York, N. Y., 1954, Vol. 1, p. 177.

(30) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **135**, 321 (1940).

(31) E. Baer and H. O. L. Fischer, *ibid.*, **128**, 491 (1939).

tively. Benson and Maruo now assign the D-terminology to an α-glycerolphosphoric acid with the spatial arrangement shown by formula XI.[21] This form has the phosphate group attached to a hydroxyl group which does not yet exist in the reference compound, D-glyceraldehyde, but is formally derivable by reduction of its carbonyl function. The basing of a new notation on such a purely formal and practically unrealized relationship does not seem desirable, particularly as no advantages are likely to be obtained by the proposed change in the established nomenclature.

Experimental

Materials.—D-α,β-Diolein was prepared by the method of Baer and Buchnea.[7] The 2'-hydroxyethylphthalimide was obtained by the procedure of Rose,[8] and recrystallized twice from water; m.p. 126.5–127.5°; reported m.p.'s 126.5–127.5°,[10,32] 126–127°[8] and 130.5.[0,9] Chloroform was dried and freed of ethanol immediately before use by distilling it over phosphorus pentoxide. Anhydrous pyridine was prepared from the best commercial grade of pyridine available, by refluxing it over barium oxide, and distilling it with the exclusion of moisture. Anhydrous quinoline was prepared from synthetic quinoline by shaking it for two days with barium oxide, and distilling the quinoline *in vacuo* under anhydrous conditions. Cyclohexane was dried with metallic sodium. The phosphorus oxychloride was purified by fractional distillation. The silicic acid was Merck Reagent Grade.

All solvents were removed under reduced pressure, and in an atmosphere of nitrogen, introduced by a capillary leak.

Dioleoyl-L-α-glycerylphosphoryl-2'-hydroxyethylphthalimide (III).—In an oven-dried 300 ml. three-necked round flask with ground joints, and equipped with an oil-sealed, mechanically driven stirrer, calcium chloride tube and dropping funnel, was placed 4.62 g. (0.03 mole) of phosphorus oxychloride. The flask was immersed in an ice-bath, and to the vigorously stirred phosphorus oxychloride was added dropwise a solution of 18.63 g. (0.03 mole) of D-α,β-diolein[8] and of 4.3 g. of quinoline in 20 ml. of cyclohexane over a period of 90 minutes. The temperature of the reaction mixture then was raised to 25° and the stirring was continued. After one hour, the temperature of the mixture was lowered to 10°, and a solution of 5.74 g. (0.03 mole) of 2'-hydroxyethylphthalimide and 8.0 g. of pyridine in 70 ml. of chloroform was added over a period of 30 minutes. After keeping the mixture at 25° for two hours, its temperature was lowered to 10°, and 0.54 ml. (0.03 mole) of distilled water was added. The mixture was allowed to stand for 30 minutes at 10° and one hour at 25°. At the end of this period it was diluted with 400 ml. of ether, filtered with suction, and the filter residue was rinsed with three 30-ml. portions of ether. The combined filtrates were washed successively with three 300-ml. portions of ice-cold 2 *N* sulfuric acid, two 200-ml. portions of a saturated sodium bicarbonate solution and two 300-ml. portions of water. To destroy the rather stable emulsions which form on washing with water, 10 ml. of ethanol was added, and the mixtures were centrifuged. The ether solution was dried with 100 g. of anhydrous sodium sulfate, filtered and concentrated under reduced pressure to a viscous oil. The residue was redissolved in 450 ml. of low-boiling petroleum ether, the solution was kept at −10° for 15 hours, and cleared by centrifugation in a refrigerated centrifuge at −10°. The solvent was distilled off under reduced pressure, and the residue was dried to constant weight in a good vacuum (0.02 mm.). The remaining L-α-(dioleoyl)-N-phthaloylcephalin, a viscous oil, weighed 24.0 g. (91.6% of theory) and was pure enough (Calcd.: N, 1.60; P, 3.55. Found: N, 1.50; P, 3.60) for the preparation of L-α-(dioleoyl)-cephalin.

To obtain analytically pure material it was treated as follows. A solution of 24.0 g. of phthaloylcephalin in 200 ml: of low boiling petroleum ether was passed through a column of silicic acid,[33] 4 cm. wide and 60 cm. high, previously wetted with petroleum ether. The column was washed with a mixture of 20 parts of ether and 80 parts of low-boiling petroleum ether (v./v.) until the effluent was free of solute. The phthaloylcephalin was recovered by washing the column exhaustively with a mixture of 20 parts of methanol and 80 parts of ether. The eluate was concentrated under reduced pressure, and the residue was freed of solvent by keeping it in a good vacuum until constant weight was reached. The remaining L-α-(dioleoyl)-N-phthaloylcephalin, a viscous and slightly colored oil, weighed 22.0 g. (84% of theory based on diolein). Investigation of the phthaloylcephalin by the paper chromatographic method of Marinetti and Stotz[13] showed it to be chromatographically homogeneous. It was found to be highly soluble in acetone, chloroform, ether, petroleum ether, glycol monomethyl ether, cyclohexane or benzene, but sparingly soluble or insoluble in methanol, ethanol or water; $[\alpha]_D$ +3.1° in chloroform (*c* 13), M_D +27.1°, $n^{24}D$ 1.4885. *Anal.* Calcd. for $C_{49}H_{80}O_{10}PN$ (874.2): C, 67.33; H, 9.22; P, 3.55; N, 1.60. Found[34]: C, 67.40; H, 9.05; P, 3.50, 3.53; N, 1.56, 1.65.

Dioleoyl-L-α-glycerylphosphorylethanolamine.—To a solution of 22.0 g. (0.0252 mole) of crude phthaloylcephalin in 300 ml. of ethylene glycol monomethyl ether, which was kept at 8° (±1°), was added dropwise with vigorous stirring 50 ml. of a 0.5 *N* aqueous solution of sodium hydroxide (0.025 mole) over a period of one hour. This was followed by the addition of a solution of 0.88 g. (0.0275 mole) of hydrazine (100%) in 0.9 ml. of water, over a period of 10 minutes. The temperature of the continuously stirred reaction mixture then was gradually raised from 8 to 60° within 2 hours, and kept at this temperature for another hour. The solvents then were distilled off under water-pump vacuum, and the residue was thoroughly dried at 0.1–0.01 mm. at a bath temperature of 40°. The residue was extracted with three 150-ml. portions of warm ether, the combined ether extracts were cleared by centrifugation, and concentrated under reduced pressure to about one-third of the original volume. To the concentrate was added 3 ml. of water, 3 ml. of methanol and 50 g. of Amberlite IRC-50 (H), and the mixture was shaken for 30 minutes. The Amberlite was filtered off, washed thoroughly with ether, and the combined filtrates were evaporated under reduced pressure. The residue was dried to constant weight in a high vacuum. The crude cephalin, weighing 18 g., was dissolved in 200 ml. of chloroform, and the solution was passed through a column of silicic acid (700 g.) of approx. 50 cm. length and 6 cm. width. The preparation of the column is described below. The column then was washed with chloroform until the effluent was free of solute. Approximately 1 l. of chloroform was required. This was followed by 2 l. of a mixture of chloroform and methanol (4:1, v./v.). The first 1000 ml. of eluate contained 5.94 g. of material low in nitrogen and phosphorus (N, 0.75; P, 3.70), the next 500 ml. of eluate contained 2.75 g. of fairly pure cephalin (N, 1.85; P, 4.20) but still gave two spots on paper chromatographic analysis. The last 500 ml. of eluate, containing pure cephalin, was evaporated under reduced pressure, and the residue was kept *in vacuo* (0.1–0.05 mm.) at a bath temperature of 35–40° until its weight was constant. The dioleoyl-L-α-glycerylphosphorylethanolamine, weighing 8.2 g., was obtained in a yield of 44% based on phthaloylcephalin; over-all yield based on diolein 40%. The L-α-(dioleoyl)-cephalin, an almost white, waxy and slightly hygroscopic material, was found to be highly soluble at room temperature (25°) in chloroform, ether or petroleum ether, readily soluble in methanol (20.2 g. per 100 ml. of solution) or ethanol (13.2 g. per 100 ml. of solution) and sparingly soluble in anhydrous acetone (2.6 g. per 100 ml. of solution). With water, it readily forms emulsions; $[\alpha]_D$ +6.0° in chloroform (*c* 7). For analysis, it was dried *in vacuo* (0.01 mm.) over phosphorus pentoxide. *Anal.* Calcd. for $C_{41}H_{78}O_8NP$ (744): C, 66.18; H, 10.57; N, 1.88; P, 4.17; iodine no., 68.0. Found[34]: C, 66.00; H, 10.47; N, 1.88, 1.90; P, 4.13, 4.13; iodine no., 66.2, 67.0.

Recovery of Oleic Acid.—The saponification of L-α-(dioleoyl)-cephalin, and recovery of the fatty acid was carried out as described for L-α-(dioleoyl)-lecithin[2]; 500.1 mg. of cephalin gave 388.2 mg. of crude oleic acid, which on

(32) S. Gabriel and H. Ohle, *Ber. chem. Ges.*, **50**, 820 (1917).

(33) To increase the rate of flow, the silicic acid was sifted through a sieve of 150 meshes per linear inch to remove particles smaller than 100 μ.

(34) The carbon and hydrogen values were obtained by combusting the phosphate in the presence of vanadium pentoxide.

distillation gave 360.5 mg. (95% of theory) of pure oleic acid, m.p. 14–15°, $n^{22}D$ 1.4580.

Distearoyl-L-α-glycerylphosphorylethanolamine (IV).—In an all-glass hydrogenation vessel of 250-ml. capacity were placed 0.15 g. of platinic oxide[35] and 25 ml. of glacial acetic acid, and the oxide was reduced to platinum black. After replacing the hydrogen with nitrogen, the acetic acid was decanted and the catalyst was washed with three 25-ml. portions of glacial acetic acid. To the catalyst was then added a solution of 1.5 g. of L-α-(dioleoyl)-cephalin in 35 ml. of glacial acetic acid, the nitrogen was replaced by hydrogen, and the hydrogenation of the unsaturated cephalin was carried out at room temperature and at a pressure of approximately 50 cm. of water. The reduction was complete in 30 minutes, with the consumption of the theoretical amount of hydrogen. After replacing the hydrogen with nitrogen, and warming the mixture to 50°, the catalyst was removed by centrifugation and washed twice with small amounts of warm acetic acid. The combined solutions were brought to dryness under reduced pressure at a bath temperature of 40–50°. The L-α-(distearoyl)-cephalin, weighing 1.5 g. (theoretical yield), was stirred for 10 minutes with 25 ml. of 25% acetic acid, and the mixture was separated by centrifugation. The precipitate was treated in the same manner successively with three 15-ml. portions of anhydrous acetone. The cephalin was freed of acetone in an air current, and was dried *in vacuo* over sodium hydroxide. For recrystallization, the cephalin was dissolved in 95 ml. of chloroform, and to the solution now was added twice its volume of methanol. The solution, after clearing by centrifugation if necessary, was set aside at room temperature until spontaneous crystallization had set in, and then was placed in an ice-box at 5° for 12 hours. The cephalin was collected with suction on a buchner funnel, washed with pure ether, and dried *in vacuo*. The L-α-(distearoyl)-cephalin weighed 1.2 g. (80% recovery). It began to sinter slightly at 130°, turned amber at about 170° and coalesced suddenly with the formation of a meniscus at 180–181°,[36] $[\alpha]D$ +6.0° in a mixture of chloroform and acetic acid (9:1 v./v., *c* 5.5); authentic L-α-(distearoyl)-cephalin,[16,17] m.p. 180–182° with sintering at about 130–135°, $[\alpha]D$ +6.0°. *Anal.* Calcd. for $C_{41}H_{82}O_8NP$ (748.1): P, 4.14; N, 1.87. Found: P, 4.15; N, 1.83.

Preparation of a Silicic Acid Adsorption Column for the Chromatographic Purification of L-α-(Dioleoyl)-cephalin.—To 700 g. of silicic acid (Merck Reagent grade) was added with stirring 0.7 l. of methanol, the slurry was filtered with suction on a buchner funnel, and the silicic acid was washed on the filter with three 300-ml. portions of methanol. The silicic acid then was suspended in 700 ml. of chloroform, and the slurry was poured into a glass column (6 cm. width and 60 cm. length) equipped at its top with a 2 l. reservoir and at its base with a stopcock and a perforated procelain plate covered with a disk of filter paper. The silicic acid was washed with chloroform until it was translucent. To promote a uniform settling of the silicic acid, the tube was occasionally gently tapped.

Acknowledgment.—The work was assisted with funds allocated by the Province of Ontario under the National Health Grants Program of the Department of National Health and Welfare. The infrared spectrum was obtained through the courtesy of Dr. G. V. Marinetti of the Department of Biochemistry and of Dr. W. B. Mason of the Atomic Energy Project of the University of Rochester School of Medicine and Dentistry, and the determination was made possible in part by funds from the United States Atomic Energy Commission.

(35) Prepared as described in "Organic Syntheses," Coll. Vol. I, 2nd edition, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 463, with the exception that the sodium nitrate was replaced by an equimolecular amount of potassium nitrate.

(36) The melting point determination was carried out in a capillary tube using an electrically heated bath of *n*-butyl phthalate and short-stem thermometers with a range of fifty degrees. The temperature of the bath was raised at a rate of 15–20° per minute up to 150°, and 4–5° thereafter.

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[CONTRIBUTION FROM THE PROCTOR & GAMBLE CO., MIAMI VALLEY LABORATORIES]

Preparation and Properties of Various Fatty Compounds Containing Lactic Acid

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The following new fatty compounds containing lactic acid have been prepared and the phase behavior of these compounds is described: O-palmitoyllactic acid, monomorphic: 1-mono-O-palmitoyllactin, dimorphic (metastable form, fleeting): 1-O-palmitoyllactyl-2,3-dilactin, monomorphic (α, stable): 1-palmitoyl-2,3-dilactyllactin, monomorphic (α, stable); tri-O-palmitoyllactin, dimorphic (α, fairly stable); 1-O-palmitoyllactyl-2,3-dipalmitin, dimorphic (unusually stable super-α). 1-Palmitoyl-2,3-dilactin, monomorphic (α, stable), has been reported previously. Of particular interest were 1-O-palmitoyllactyl-2,3-dilactin, 1-palmitoyl-2,3-dilactyllactin and 1-palmitoyl-2,3-dilactin. These triglycerides, each with one long and two short chains, showed stable α-forms: likewise, having two unesterified hydroxyl groups in the molecule, they possessed surface activity of the same order of magnitude as monoglycerides such as 1-monoölein.

Introduction

A series of lactic acid glycerides of known structure was made as part of a continuing program for the preparation of new compounds of potential value as components of edible products. In general, the synthetic approach was the same as that described by Goldblatt and co-workers.[1] Lactic acid was introduced into the glyceride molecule as the O-benzyllactic acid derivative. The benzyl group was subsequently removed by hydrogenolysis to yield the final product or to render the hydroxyl group of the lactic acid radical available for further reaction. The phase behavior of these compounds was compared with that of related but more familiar fatty compounds. Because of the hydrophilic groups, these compounds were also tested for interfacial activity in fat–water systems.

Experimental

Synthesis.—The following are typical preparations of the lactic acid glycerides and related compounds not previously described in the literature.

O-Palmitoyllactic Acid.—A solution of 27 g. (0.15 mole) of benzyl lactate, prepared as described by Fein and Fisher,[2] and 18 g. of pyridine in 150 ml. of dry chloroform was chilled in ice, and 41.2 g. (0.15 mole) of palmitoyl chloride was added dropwise with swirling. After standing for 4 days at room temperature, the solution was diluted with ether, water-washed three times, dried over sodium sulfate,

(1) L. A. Goldblatt, D. A. Yeadon and M. Brown, THIS JOURNAL, **77**, 2477 (1955).

(2) M. L. Fein and C. H. Fisher, *J. Org. Chem.*, **15**, 530 (1950).