The authors express their sincere gratitude to Dr. E. Ochiai, Professor Emeritus of University of Tokyo, for his kind advices, and to Dr. T. Kariyone, the director of our Institute, and to Dr. M. Kusami, Dean of Showa Phamaceutical College, for their encouragements. They are also indebted to Dr. T. Oba and Mr. G. Kawabata for IR-spectroscopy, and to members of Analytical Centres of University of Tokyo and Kowa-Co. Ltd. for elemental microanalysis, and to Miss N. Sato for her technical assistance in the experiment.

Summary

The nucleophilic activity of methyl group in 3,6-dimethylpyridazine 1-oxide (I) and monomethylpyridazine 1-oxides (II) with benzaldehyde was studied. Their corresponding (β-hydroxyphenethyl)pyridazine 1-oxides and styryl compounds were gained. From the results, it may be deduced as follows. (i) The reactivity of 3- and 6-methyl groups in I were almost same, different from the cases of 3,6-dichloropyridazine 1-oxide. (ii) 4- and 6-methyl groups in II were similarly reactive, but 5-methyl was more active, and 3-methyl less active than fore-mentioned two methyl groups. These styryl compounds that were produced respectively, were catalytically hydrogenated with Pd-C to corresponding phenethylpyridazine 1-oxides.

(Received April 1, 1963)

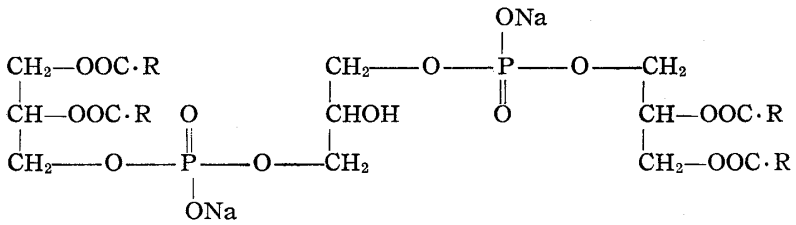
[Chem. Pharm. Bull. 11 (9) 1150 ~ 1156]

UDC 576.8.097[616-002.6-078] : 612.398.145

188. Keizo Inoue, Yasuji Suhara, and Shoshichi Nojima : On the Cardiolipin Analogues. Syntheses of Dipalmitoyl-D,L-α-glycerylphosphoryl-propanol Sodium Salt and Bis(dipalmitoyl-D,L-α-glyceryl-phosphoryl)-1,3-propanediol Disodium Salt.

(*Faculty of Pharmaceutical Sciences, University of Tokyo*[*1])

Cardiolipin, the phosphatide as a constituent of the Wasserman antigen in the diagnosis of syphilis, was first isolated from beef heart by Pangborn in 1941.[1] It was then characterized as a complex phosphatidic acid derivatives.[2] Similar derivatives with different fatty acids have been found in many animals and vegetable tissues and are, for the time being, recognized as one of the important components among the phosphatides in the tissues. The chemical composition and structure of this phosphatide were recently reinvestigated by several workers and the following new chemical structure was proposed.[3]



R·CO-=Unsaturated fatty acid residue

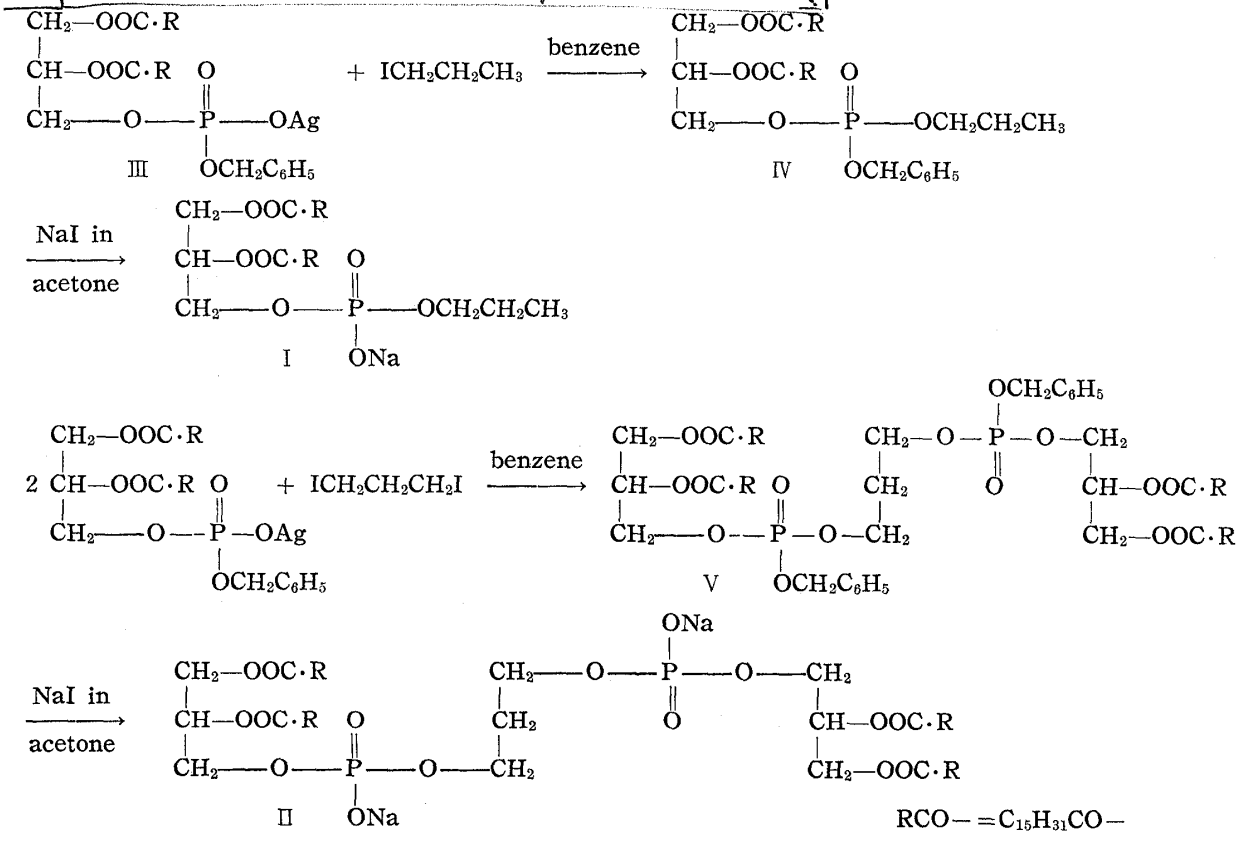
[*1] Hongo, Tokyo (井上圭三, 須原康次, 野島庄七).

1) M.C. Pangborn : Proc. Soc. Exptl. Biol. Med., **48**, 484 (1941).
2) *Idem* : J. Biol. Chem., **168**, 351 (1947).
3) M.G. Macfarlane : Nature, **182**, 946 (1958) and **183**, 1808 (1959); M. Faure, M.J. Morelec-Coulon : Ann. Inst. Pasteur, **91**, 537 (1956); A.A. Benson, E.H. Strickland : Biochim. et Biophys. Acta, **41**, 328 (1960).

The present authors have undertaken to attempt the syntheses of cardiolipin analogues and their derivatives in order to have some knowledge in the relationship between the chemical structure of cardiolipin and its serological specificity.

The syntheses of naturally occurring various phosphatides, such as phosphatidylcholine, -ethanolamine or -serine, have been successfully achieved by several groups of investigators, especially by those of Baer,[4] Malkin,[5] Verkade,[6] and Van Deenen.[7] A prerequisite to the elaboration of phosphatides syntheses is an efficient method to form the phosphodiester linkage similar to the naturally occurring phosphatides. In principle, the synthesis of such a linkage may be approached in the following two ways. In the first approach, two compounds, each bearing a hydroxyl group, for example, an α,β-diglyceride on the one hand and choline, ethanolamine or serine derivatives on the other, are condensed in successive steps to a bifunctional phosphorylating agent. This approach has been used with considerable success in syntheses of phosphatidylcholine-ethanolamine and other phosphatides by Baer and his collaborators. The second approach to the phosphodiester linkage synthesis on which the very recent work of mixed acid phosphatides was based is to form a protected trialkyl phosphate by reaction of silver salt of dialkyl hydrogen phosphate with alkyl halide, followed by removal of the unnecessary group.[7] As Malkin[5] and Stanacev and Kates[8] already pointed out in the syntheses of phosphatidic acid derivatives, the latter method seemed more convenient in synthesizing the phosphatides from several reasons. The main reason is

The chemical synthetic pathways are shown below.



RCO– = $C_{15}H_{31}CO$–

4) For example. E. Baer: Can. J. Biochem. Physiol., **34**, 288 (1956).
5) For example. T. Malkin, T. H. Bevan: The Synthesis of Phospholipids in "Progress in the Chemistry of Fats and Other Lipids," Vol. IV, p. 97 (1957), Pergamon Press, London.
6) J. W. Gielkens, M. A. Hoelnagel, L. J. Stegerhoek, P. E. Verkade: Rec. Trav. Chim., **77**, 656 (1958).
7) G. H. De Haas, L. L. M. Van Deenen: Rec. Trav. Chim., **80**, 951 (1961).
8) N. Z. Stanacev, M. Kates: Can. J. Biochem. Physiol., **38**, 297 (1960).

that the bifunctional phosphorylating agent used in the first method inevitably produces considerable amount of undesirable symmetrical bis-phosphatidic acid as by-product.

This paper deals with the syntheses of dipalmitoyl-D,L-α-glycerylphosphorylpropanol sodium salt (I) and of bis(dipalmitoyl-D,L-α-glycerylphosphoryl)-1,3-propanediol disodium salt (II) according to the latter approach described above.

As the starting material in synthesizing both compounds (I) and (II), dipalmitoyl-D,L-α-glycerophosphoric acid benzyl ester silver salt (III) was prepared through the 2-procedures by Stanacev and Kates,[8] originally reported by Verkade and his collaborators,[6] in the following sequence: glycerol ⟶ D,L-isopropylideneglycerol ⟶ D,L-3-iodo-1,2-propanediol ⟶ dipalmitoyl-D,L-α-glycerophosphoric acid dibenzyl ester ⟶ dipalmitoyl-D,L-α-glycerophosphoric acid benzyl ester sodium salt ⟶ III.

Dioleoyl-D,L-α-glycerophosphoric acid propyl ester was reportedly synthesized by Kuhn and Klesse[9] through a different synthetic route from that of the present study.[9] In this study, III was condensed with propyl iodide in dry benzene to yield dipalmitoyl-D,L-α-glycerylphosphorylpropanol benzyl ester (IV). When the residue, obtained after removal of silver iodide from the reaction mixture and successive evaporation of the solvent from the filtrate, was checked on silica gel thin layer chromatogram (TLC), several minor components above and below the main spot were detected. The purification of this material was carried out with silicic acid column chromatography and IV was obtained in a yield of 26%.

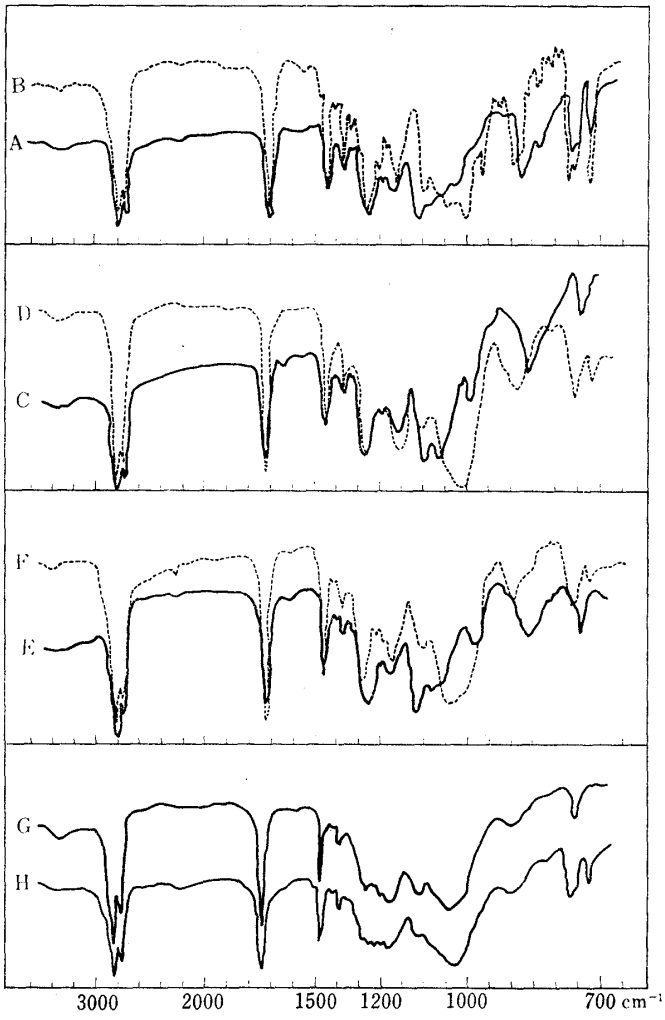


Fig. 1. Infrared Spectra of Synthetic Phosphatides

A Nihon Bunko IR-S (NaCl prism) was used. KBr disk. Ordinate : Transmission, %. Abscissa : Wave number

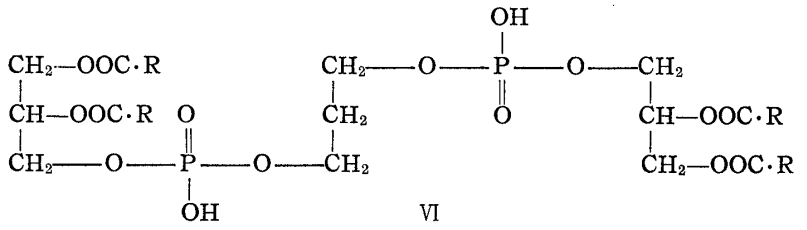
A : Dipalmitoyl-D,L-α-glycerophosphoric acid benzyl ester sodium salt
B : Dipalmitoyl-D,L-α-glycerophosphoric acid dibenzyl ester
C : Dipalmitoyl-D,L-α-glycerylphosphorylpropanol sodium salt (I)
D : Dipalmitoyl-D,L-α-glycerylphosphorylpropanol monobenzyl ester (IV)
E : Bis(dipalmitoyl-D,L-α-glycerylphosphoryl)-1,3-propanediol disodium salt (II)
F : Bis(dipalmitoyl-D,L-α-glycerylphosphoryl)-1,3-propanediol dibenzyl ester (V)
G : Bis(dipalmitoyl-D,L-α-glycerylphosphoryl)-1,3-propanediol (VI)
H : Dipalmitoyl-D,L-α-glycerophosphoric acid benzyl ester

9) R. Kuhn, P. Klesse : Z. Physiol. Chem., **312**, 214 (1958).

In general, the removal of benzyl group from a phosphotriester as described above is smoothly conducted by two kinds of procedures, *i.e.* catalytic hydrogenation for entire debenzylation[10] and monodebenzylation with sodium iodide.[11] In the present experiment, the latter procedure seemed to have an advantage in giving sodium salt of the phosphatides which is more stable than acidic form, especially in the cases of the acidic phosphatides such as phosphatidic acid, phosphatidylglycerol or cardiolipin. From this reason, IV was debenzylated with sodium iodide in boiling acetone to give I in a yield of 60.2%. The overall yield from III was 15.7%.

In order to synthesize the dibenzyl ester of bis(dipalmitoyl-D, L-α-glycerylphosphoryl)-1,3-propanediol (V), 1,3-diiodopropane was condensed with III in the similar manner as described above. In this case, however, the reaction proceeded more slowly with accompanied change of color of the solution to red-brown. The dibenzyl ester (V) was obtained in a yield of 16.6%, after purification with silicic acid column chromatography. This purified material showed, in spite of the good agreement of the analytical data with the theoretical values and typical trialkyl phosphate patterns in infrared spectrum (Fig. 1), the main intense spot (Rf 0.65) was followed by a very weak spot (Rf 0.60) on silica gel TLC and was difficult to be separated from the minor spot even by further chromatography or solvent fractionation.

Debenzylation of V was also successful carried out with sodium iodide in boiling acetone to obtain bis(dipalmitoyl-D, L-α-glycerylphosphoryl)-1, 3-propanediol disodium salt (II). Upon purification of this substance with silicic acid column chromatography was obtained a material which was identified as bis(dipalmitoyl-D, L-α-glycerylphosphoryl)-1,3-propanediol (VI), a cation-free form of II, by microanalysis of C, H and P, infrared spectra (G and H in Fig. 1) and acid titration with potassium hydroxide using



VI

phenolphthalein. This result is of interest in view of the experiments recently reported by Rathbone[12] who demonstrated an exchange of lipid Na^+ or K^+ with H^+ ions during silicic acid column chromatography in the case of human brain phosphatidylserine. While silicic acid columns have, in general, found the widest application for the chromatographic separation of phosphatides, it should be considerably important to know, from both the above experimental results and the results of Marinetti, *et al.*[13] and Rathbone, that some kind of interactions exists against the acidic lipids such as cardiolipin, phosphatidylglycerol, phosphatidic acid or sulphatides similar to the case of phosphatidylserine. The present experiment clearly demonstrated the fact of exchange, if not quantitatively, of Na^+ of II with H^+ ions, presumably derived from silicic acid. Further investigation of this problem using synthetic materials is now in progress.

EXPERIMENTAL: The infrared spectra of I, II and sodium salt of dipalmitoyl-D,L-α-lgycerophosphoric acid benzyl ester, their benzyl derivatives and their cation-free acids were shown in Fig. 1. When comparing the spectra of the salts of these synthetic phosphatides with those of benzyl derivatives or of the cation-free forms, some interesting

10) E. Baer : J. Biol. Chem., **189**, 235 (1951).
11) L. W. Hessel, I. D. Morton, A. R. Todd, P. E. Verkade : Rec. Trav. Chim., **73**, 150 (1953).
12) L. Rathbone : Biochem. J., **85**, 461 (1962).
13) G. W. Marinetti, J. Erbland, E. Stotz : Biochim. et Biophys. Acta., **30**, 41 (1958).

relationships are to be noted. In the absorption region 900~1125 cm^{-1} (probably P-O-C (alkyl)), both benzyl derivatives (B, D and F in Fig. 1) and free acids (G and F) showed the strongest absorption peak at 1020~1035 cm^{-1}, while sodium salts at ca. 1100cm^{-1} (A, C and E). On the other hand, in the region 1125~1300 cm^{-1}, salt forms as well as benzyl derivatives gave two characteristic strong absorption peaks, whereas several weak absorption bands appeared in the case of free acids.

As possible substitutes for cardiolipin in the serodiagnosis of syphilis, both I and II are under investigation by Drs. H. Tomizawa and R. Murata in Department of Bacteriology II, National Institute of Health, Tokyo. Preliminary experiment by slide flocculation test (VDRL) indicated that II was fairly reactive as a cardiolipin substitute while I showed a rather weak activity.

Methods and Materials

Palmitic acid was purchased and purified with repeated recrystallization from Me_2CO. The purity of the material was checked by gas liquid chromatography. The contamination of other fatty acids in this preparation was found to be less than 1%. Propyliodide was obtained commercially. 1,3-diiodopropane was prepared according to the method of Landauer and Rydon[14] : $b.p_{0.8}$ 54°. This material was redistilled just before use.

Thin-layer chromatography was performed*2 on Silica Rider (Daiichi Pure Chemicals Co., Ltd. Tokyo) with the following solvent systems; $CHCl_3$ only (1), $CHCl_3$–CH_3OH (100:1) (2), $CHCl_3$–CH_3OH (50:1) (3), $CHCl_3$–CH_3OH–H_2O (100:15:1) (4), $CHCl_3$–CH_3OH–H_2O (65:25:4) (5) and $CHCl_3$–CH_3OH–H_2O (40:10:0.5) (6). In this report, the Rf values of the compounds found for each of these solvent systems are represented with the abbreviations Rf_1, Rf_2, Rf_3, Rf_4, Rf_5 and Rf_6, respectively.

The silicic acid for column chromatography was from Mallinckrodt 100 mesh (powder), analytical reagent and washed several times with Me_2CO and activated at 110° for 1 hr. Hyflosupercel was washed with H_2O, EtOH and Me_2CO successively and dried in air.

Phosphorus was determined according to King.[15] Ester-determination was followed by the procedure of Rapport and Alonzo.[16]

Experimental

Dipalmitoyl-D,L-α-glycerophosphoric Acid Benzyl Ester Silver Salt (III)——The preparation of dipalmitoyl-D,L-α-glycerophosphoric acid benzyl ester silver salt (III), starting from glycerol, was carried out according to the route described in the text. Yields and physical data for a series of the compounds are as follows : glycerol → D,L-isopropylideneglycerol, $b.p_{12}$ 83~83.5° (94% of theory) → D,L-3-iodo-1,2-propanediol, m.p. 42~45° (58.4% of theory) → Dipalmitoyl-D,L-α-glycerophosphoric acid dibenzyl ester, m.p. 51~53° (68% of theory) → Dipalmitoyl-D,L-α-glycerophosphoric acid benzyl ester sodium salt, m.p. 160~165° (90% of theory) → III, m.p. 120° (52% of theory).

Dibenzylphosphate Silver Salt——The preparation of the silver salt of dibenzyl phosphate was carried out by monodebenzylation of tribenzyl phosphate with NaI.[17] The tribenzyl phosphate was prepared according to the method of Zervas and Dilaris with a slight modification in the following manner.[18] A sample of 7.73 g. (18.5 mmole) of dried silver phosphate was condensed with 10 g. of freshly distilled benzylchloride in 200 ml. of dry benzene under reflux for 10 hr. At the end of the reaction, the precipitate was filtered off, the supernatant was evaporated to dryness and the residue crystallized after storing for several days in a desiccator, m.p. 60~61.5°, 2.3 g. (38.4% of theory).

Dipalmitoyl-D,L-α-glycerylphosphorylpropanol Benzyl Ester (IV)——A sample of 2.0 g. (2.36 mmole) of dipalmitoyl-D,L-α-glycerophosphoric acid benzyl ester silver salt (III) dried over P_2O_5 *in vacuo* at 40° for 12 hr. was dissolved in 100 ml. of boiling, anhydrous and thiophene-free benzene, and to the solution

*2 The authors wish to thank Dr. T. Furuya of Dept. of Pharmacognogy, University of Tokyo, for many helpful discussion and suggestions during the performance of TLC.

14) R. Landauer, H. Rydon : J. Chem. Soc., 2229 (1953).
15) E. J. King : Biochem. J., **26**, 292 (1932).
16) M. M. Rapport, N. Alonzo : J. Biol. Chem., **217**, 193 (1955).
17) W. Lossen, A. Koehler : Ann. **262**, 213 (1891).
18) L. Zervas, I. Dilaris : J. Am. Chem. Soc., **77**, 5354 (1955).

was added dropwise a solution of 0.80 g. (4.72 m mol.) of PrI in 10 ml. of dry benzene, and the solution was boiled under reflux and stirred with exclusion of light for 2 hr. Immediately after the addition of one drop of PrI solution, the precipitation of yellow colored AgI began. During the reaction period of 2 hr., the aliquots of the supernatant of the reaction mixture were drawn every thirty min. and were checked on silica gel TLC. At the end of the addition of the PrI solution, a new spot (Rf_2 0.5) was already detected on TLC, whereas the silver salt remained at the original point (Rf_2 0). After 30 min., the intensity of the new spot (sprayed with Rhodamine 6G) reached maximum and remained unchanged during the period of 2 hr. The reaction mixture was then cooled to room temperature, filtered through a glass filter to remove precipitates of AgI (82% of theory). The faintly yellow colored filtrate was evaporated to dryness under reduced pressure (rotatory evaporater) on a bath at 30°. The waxy faint yellow solid residue, was dried over P_2O_5 in a vacuum desiccator overnight. For the purification of benzyl ester of the phosphatide, a solution of the residue in 100 ml. of freshly distilled $CHCl_3$ was passed through a column of 50 g. of silicic acid mixed with 25 g. of Hyflosupercel (2.5×30.0 cm.), and eluted with $CHCl_3$ collecting 50 ml. of the effluent in each tube. After emerging of several minor components in the faster moving fractions, the main fractions were collected from Tubes 7 to 14 to give 0.493 g. of the residue after evaporation of the solvent. The residue was again dissolved in $CHCl_3$, the solution was filtered and the filtrate was evaporated to dryness *in vacuo*. Dipalmitoyl-D,L-α-glycerylphosphorylpropanol benzyl ester (IV) was obtained as a colorless, odorless waxy material. Yield, 26% of theory; m.p. 25°. It is readily soluble in EtOH, benzene, Me_2CO or $CHCl_3$. This material gave only one spot on silica gel TLC with 10% H_2SO_4, Rf_1 0.1 and Rf_2 0.5. *Anal.* Calcd. for $C_{45}H_{81}O_8P$ (781.08): C, 69.16; H, 10.38; P, 3.97. Found: C, 69.12; H, 10.52; P, 3.95.

Dipalmitoyl-D,L-α-glycerylphosphorylpropanol Sodium Salt (I)——Debenzylation of 170 mg. (0.215 m mol.) of IV was carried out by refluxing for 4.5 hr. the solution of the benzyl ester (IV) in 3 ml. of dry Me_2CO with 59 mg. (0.393 m mol.) of NaI which was beforehand dried over P_2O_5 at 130° for 24 hr. At the end of the reaction, the reaction mixture was cooled in an ice box to give colorless precipitates which was filtered through a sintered glass filter. The precipitate was washed several times with a small amount of dry Me_2CO and again dissolved in 3 ml. of Me_2CO with warming and cooled to reprecipitate. The sodium salt of dipalmitoyl-D,L-α-glycerylphosphorylpropanol (I) was obtained as a colorless waxy material. Yield, 93 mg. (60.2% of theory); m.p. 200°. The substance is readily soluble at room temperature in Et_2O, benzene or $CHCl_3$, only slightly soluble in EtOH but insoluble in Me_2CO. This substance gave a single one spot on silica gel TLC with 10% H_2SO_4. *Anal.* Calcd. for $C_{38}H_{74}O_8PNa$ (712.95): C, 64.01; H, 10.46; P, 4.34. Found: C, 63.80; H, 10.42; P, 4.10.

Bis(dipalmitoyl-D,L-α-glycerylphosphoryl)-1,3-propanediol Dibenzyl Ester (V)——A sample of 3.37 g. (3.9 m mol.) of III was dissolved in 10 ml. of boiling, anhdrous and thiophene free benzene and to the solution was added dropwise within a period of 15 min. a solution of 0.35 g. (1.2 m mol.) of freshly distilled colorless 1,3-diiodopropane in 10 ml. of dry benzene. After the addition of the solution, yellow precipitates gradually deposited. The reaction mixture was refluxed and stirred in the dark for additional 5 hr. During this period, the progress of the reaction was checked similarly to as described above. The amount of the main component (Rf_2 0.15) reached a maximum within 3 hr. and further increase of the amount of this material was not observed during the period of the additional 2 hr.

After cooling, the reaction mixture was centrifuged off to remove a yellowish brown precipitate of AgI. The resulting clear red brown supernatant was evaporated to dryness under reduced pressure (rotatory evaporator) on a bath at 40°. The waxy solid residue, colored brown, was dried oved P_2O_5 in a vacuum desiccator overnight. This material showed 5 to 7 spots on TLC, the main component giving Rf_1 0.0, Rf_2 0.1~0.15 or Rf_3 0.6~0.7.

To isolate purer main component, a solution of the residue in 150 ml. of freshly distilled $CHCl_3$ was passed through a column of 50 g. silicic acid mixed with 25 g. of Hyflosupercel (2.5×30 cm.). The following volumes of solvents were applied for elution: $CHCl_3$ 350 ml., $CHCl_3$-CH_3OH (100:0.5) 200 ml. and $CHCl_3$-CH_3OH (100:1) 400 ml. and each 50 ml. per tube of eluate was taken. The main component on TLC was already detected in the effluents of the second solvent but they were contaminated with several by-products. The tubes 15 and 16 eluted with the third solvent were collected and the solvent was evaporated to dryness under reduced pressure. The residue was again dissolved in $CHCl_3$, the solution was filtered and the filtrate was evaporated to dryness. Bis(dipalmitoyl-D,L-α-glycerylphosphoryl)-1,3-propanediol dibenzyl ester (III) was obtained after drying over P_2O_5 at room temperature overnight. Yield 0.30 g. (16.6% of theory), m.p. 40°. It was negative for Beilstein's halogen test. It is readily soluble in benzene, $CHCl_3$ or Et_2O but slightly soluble in Me_2CO. On TLC of silica gel, this substance gave a single spot (Rf_3 0.65) with Rhodamine 6G. *Anal.* Calcd. for $C_{87}H_{154}O_{16}P_2$ (1518.06): C, 68.83; H, 10.23; P, 4.08. Found: C, 68.55; H, 10.39; P, 3.96.

Bis(dipalmitoyl-D,L-α-glycerylphosphoryl)-1,3-propanediol Disodium Salt (II)——A sample of 200 mg. (0.13 m mol.) of V was debenzylated by refluxing the solution of V in 3 ml. of dry Me_2CO with 59 mg. (0.393 m mol.) of dried NaI. The clear boiling solution turned cloudy after 30 min., and 1 hr. later a white resinous material deposited to the bottom of the flask. After 4 hr. refluxing, the reaction mixture was rapidly filtered through a glass filter to remove a yellow colored Me_2CO filtrate. The

remaining solid was treated several times with dry Me_2CO and finally dissolved in 0.5 ml. of $CHCl_3$, precipitating from the solution with 3 ml. of Me_2CO. Bis(dipalmitoyl-D,L-α-glycerylphosphoryl)-1,3-propanediol disodium salt (II) was obtained as a colorless powder. Yield, 138 mg. (73.0% of theory); m.p. 220~225°. This material is soluble in $CHCl_3$ but insoluble in Et_2O, EtOH or Me_2CO. Rf_5 0.86 and Rf_6 0.70. *Anal.* Calcd. for $C_{73}H_{140}O_{16}P_2Na_2$ (1381.80): C, 63.45; H, 10.21; P, 4.48. Found: C, 63.29; H, 10.19; P, 4.30.

Bis(dipalmitoyl-D,L-α-glycerylphosphoryl)-1,3-propanediol (VI)——A sample of 50 mg. of II was dissolved in 5 ml. of $CHCl_3$ and placed on a silicic acid column (silicic acid 2 g. and Hyflosupercel 1 g., 1×13 cm.), and eluted with $CHCl_3$-CH_3OH (10:1), collecting 5 ml. in each tube. The third tube was taken and the solvent was evaporated to dryness. The residue was again dissolved in 0.5 ml. of $CHCl_3$, reprecipitated from the solution with 3 ml. of dry Me_2CO. The precipitate, after centrifuging and discarding the supernatant, was dried over P_2O_5 *in vacuo* to give 13 mg. of colorless powder (26% of theory), m.p. 60~65°. Rf_6 0.83 (cf. Rf_6 of II 0.7). *Anal.* Calcd. for $C_{73}H_{142}O_{16}P_2$ (1337.82): C, 65.53; H, 10.69. Found: C, 65.75; H, 10.56.

Equivalent weight: Calcd.: 668.91, Found: 662.28 (A solution of 0.977 mg. of VI in 3 ml. of a mixture of $CHCl_3$-CH_3OH (2:1) was titrated using phenolphthalein as a indicator at room temperature with 0.1*N* aqueous solution of KOH (F=0.9968), 14.8 λ of which was consumed).

The authors thank Professor D. Mizuno, University of Tokyo, for his interest and helpful suggestions throughout the course of this work.

They are indebted to Misses N. Ninomiya and F. Suzuki for infrared spectral measurements, to Missess K. Hayashi and H. Yamanouchi, Institute of Applied Microbiology, and Dr. E. Kimura and co-workers for microanalysis.

This work was supported partly by a Grant in aid for Scientific Research provided by the Ministrtry of Education.

Summary

Dipalmitoyl-D,L-α-glycerylphosphorylpropanol sodium salt (I) and bis(dipalmitoyl-D,L-α-glycerylphosphoryl)-1,3-propanediol disodium salt (II) were synthesized by the condensation of the silver salt of dipalmitoyl-D,L-α-glycerophosphoric acid benzyl ester (III) with alkyl iodide, followed by debenzylation with sodium iodide. It was found that II was converted to an acid form upon silicic acid column.

(Received April 5, 1963)