Synthetic and Structural Investigations on 3-Phosphatidyl-1′-(3′-*O*-L-lysyl)glycerol*

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ABSTRACT: The synthesis is described of the lysyl ester of phosphatidylglycerol in the same stereochemical configuration as that of the natural compound isolated from *Staphylococcus aureus*. Starting from 2,3-isopropylideneglycerol, 3-(di-*N*-*t*-butoxycarbonyl)-L-lysyl-2-*O*-*t*-butylglycerol 1-iodohydrin was prepared *via* eight intermediates. The latter product was allowed to react with the silver salt of 1-oleoyl-2-palmitoylglycerol 3-(*O*-benzyl)phosphate to give the corresponding phosphotriester. Anionic debenzylation and treatment with hydrogen chloride yielded 1-oleoyl-2-palmitoylglycerol-3-phosphoryl-1′-(3′-*O*-L-lysyl)glycerol as the dihydrochloric acid salt. The synthetic compound and the natural lysyl ester of phosphatidylglycerol from *S. aureus* had closely similar physical and chemical properties.

Enzymic hydrolyses with phospholipase A, phospholipase C, and phospholipase D revealed that the two substances were completely identical in stereochemical configuration.

Some nitrogen-containing phospholipids from bacteria were characterized by Macfarlane (1962, 1964) and Houtsmuller and van Deenen (1963, 1965) as *O*-amino acid esters of phosphatidylglycerol. Several amino acids were found in this class of phospholipids, *e.g.*, glycine (Vorbeck and Marinetti, 1965), alanine (Macfarlane, 1962), ornithine (Houtsmuller and van Deenen, 1963), and lysine (Macfarlane, 1964; Houtsmuller and van Deenen, 1965).

The chemical synthesis of this type of phospholipids was undertaken by three research groups. Bonsen *et al.* (1965) reported the synthesis of a racemic alanyl ester of phosphatidylglycerol, starting from the silver salt of (*O*-benzyl)phosphatidic acid and the alanyl ester of glycerol iodohydrin protected with appropriate blocking groups. Debenzylation followed by treatment with hydrogen chloride furnished the alanyl ester of phosphatidylglycerol. Baer and Jagannadha Rao (1965, 1966) synthesized the glycyl and alanyl esters of phosphatidylglycerol, by a condensation reaction of 1,2-distearoylglycerol, azidoacyl-2-benzylglycerol, and phenylphosphoryl dichloride. The end products were obtained by a simultaneous reduction of the azido group and catalytic hydrogenolysis of the phenyl and benzyl groups. In these compounds, only the phosphatidyl moiety was in the correct stereochemical configuration. Bergelson and Molotkovsky (1966) described the synthesis of two optically active, completely saturated, epimers of 3-phosphatidyl(L-alanyl)glycerol, starting from the silver salt of (*O*-benzyl)phosphatidic acid and the alanyl ester of the isomeric glycerol iodohydrins with appropriate blocking groups. Catalytic hydrogenolysis of the condensation products provided the L-alanyl esters of 3-phosphatidyl-3′-glycerol and of 3-phosphatidyl-1′-glycerol (stereochemical numbering according to Hirschmann, 1960).

Houtsmuller and van Deenen (1965) succeeded in the isolation and characterization of the pure lysyl ester of phosphatidylglycerol from *Staphylococcus aureus*, which turned out to be 3-phosphatidyl-1′-glycerol esterified with L-lysine at one of the free hydroxyl functions.

The purpose of this work was to establish by synthesis the structure and stereochemical configuration of the natural product. For the synthetic lysyl ester first preference was given to the compound with the amino acid at the 3′ position of the free glycerol moiety. The choice of the fatty acid composition was determined by the fact that phospholipids with unsaturated fatty acids have better manageable properties in enzymic degradations (the natural product has mainly branched iso and anteiso C_{15} acids).

The synthesis of the starting material (X) was carried out as outlined in Scheme I. This product (X) was allowed to react with the silver salt of 1-oleoyl-2-palmitoylglycerol 3-(*O*-benzyl)phosphate (XI) to give the fully protected phosphotriester XII. Removal of the benzyl group by anionic debenzylation and splitting off of the two *t*-butoxycarbonyl groups and the *t*-butyl group with anhydrous hydrogen chloride furnished, after recrystallization, pure 1-oleoyl-2-palmitoylglycerol-3-phosphoryl-1′-(3′-*O*-L-lysyl)glycerol (XIV) as the dihydrochloric acid salt.

Results and Discussion

The chemical and physical properties of the synthetic compound were compared with the lysyl ester of phosphatidylglycerol from *S. aureus*.

Analytical Data. The elemental analysis of the

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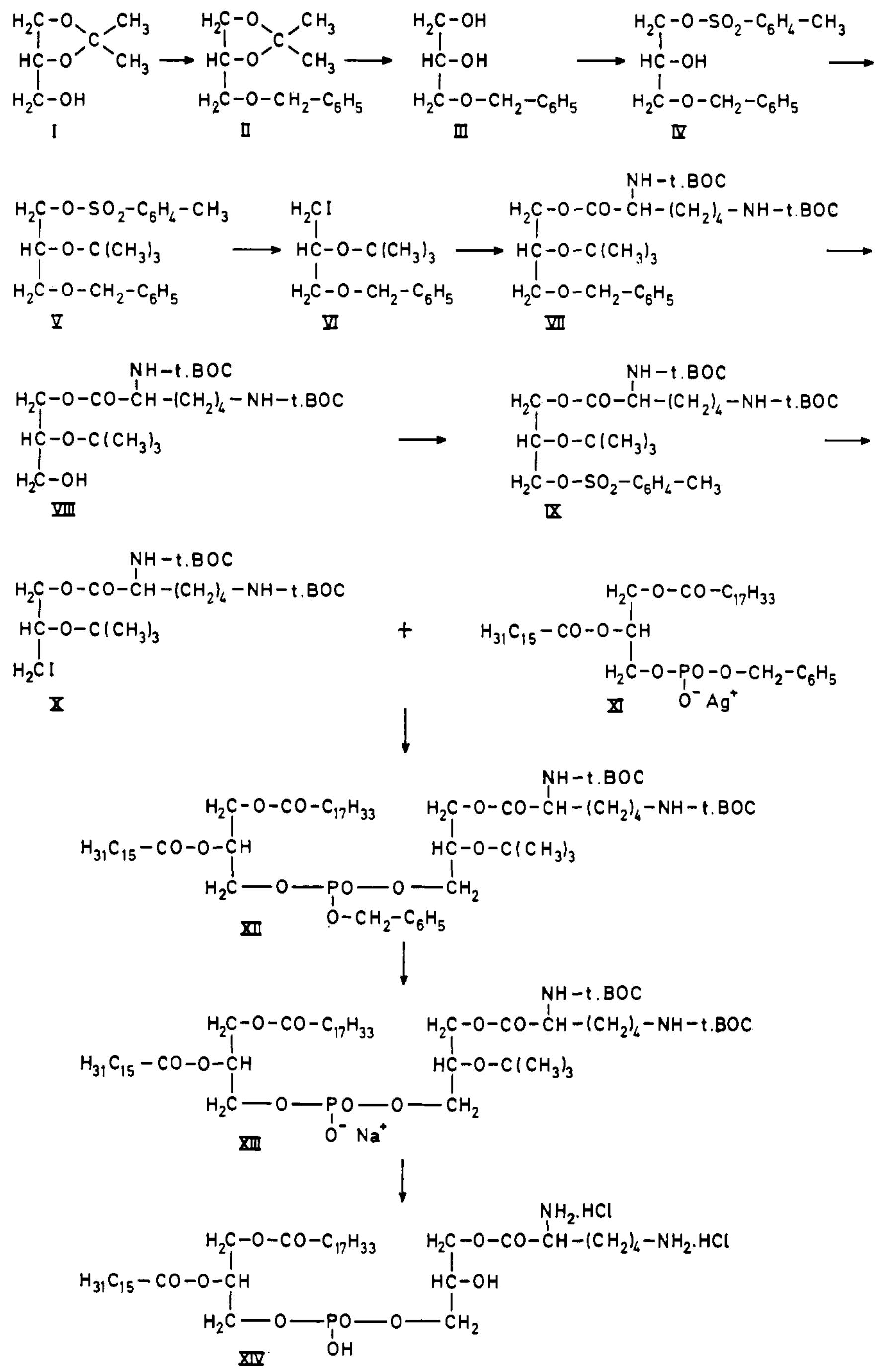
SCHEME I: Synthesis of 1-Oleoyl-2-palmitoylglycerol-3-phosphoryl-1′-(3′-*O*-L-lysyl)glycerol.



synthetic product agrees very well with the calculated values (see Experimental Section) and is comparable with that of the natural specimen. Gas chromatographic analysis of the fatty acids of the synthesized lysyl ester revealed a ratio of palmitic acid to oleic acid of 1.01. Houtsmuller and van Deenen (1965) reported for the isolated compound mainly branched saturated C_{15} fatty acids of the iso and anteiso types.

Chromatography. The synthetic compound and the natural product each give a single spot with identical R_F values on silica-impregnated paper in the solvent system of Marinetti *et al.* (1957), and on thin layer chromatograms on silica gel G developed in chloroform–methanol–water (65:25:4, v/v). The compounds were detected by means of the ninhydrin reagent, the molybdate reagent, the tricomplex staining method according to Bungenberg de Jong (1961) (red spots on paper chromatograms) and sulfuric acid (only on thin layer plates). Neither the synthetic nor the natural lysyl ester of phosphatidylglycerol produced a colora-

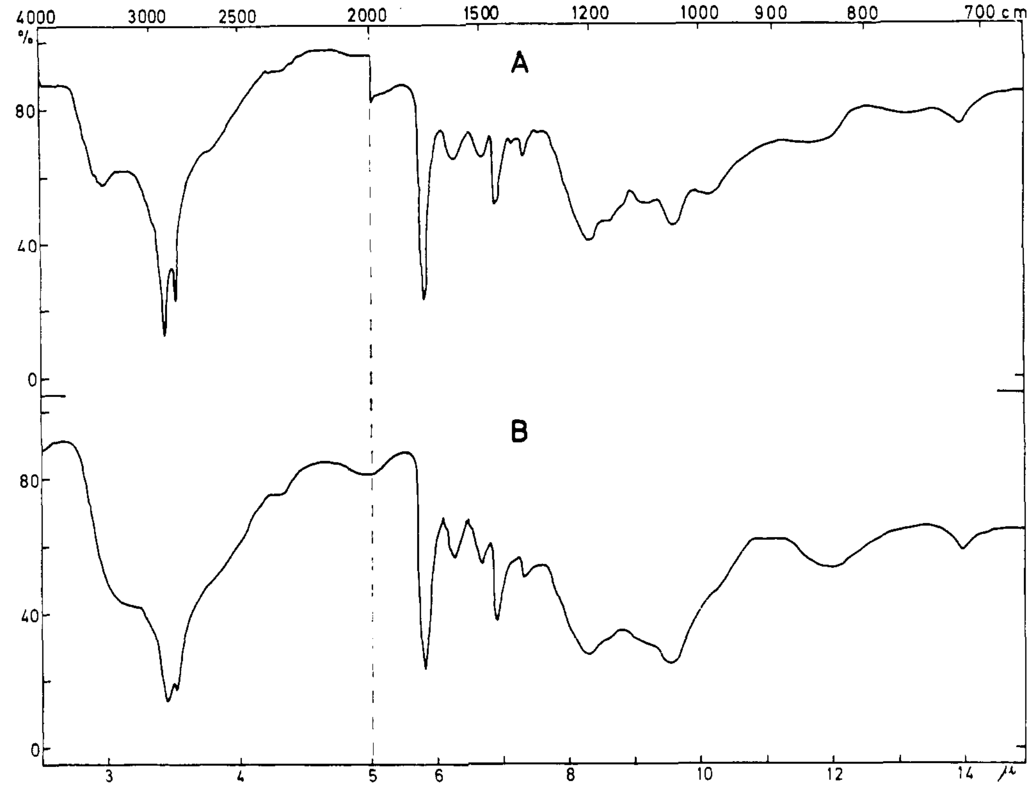


FIGURE 1: Infrared spectra. Both samples were spread as a thin film on a KBr disk. Ordinate, per cent transmission; abcissa, wavelength. (A) 1-Oleoyl-2-palmitoylglycerol-3-phosphoryl-1′-(3′-*O*-L-lysyl)glycerol. (B) Lysyl ester of phosphatidylglycerol isolated from *S. aureus.*

tion with the periodate–Schiff reagent.

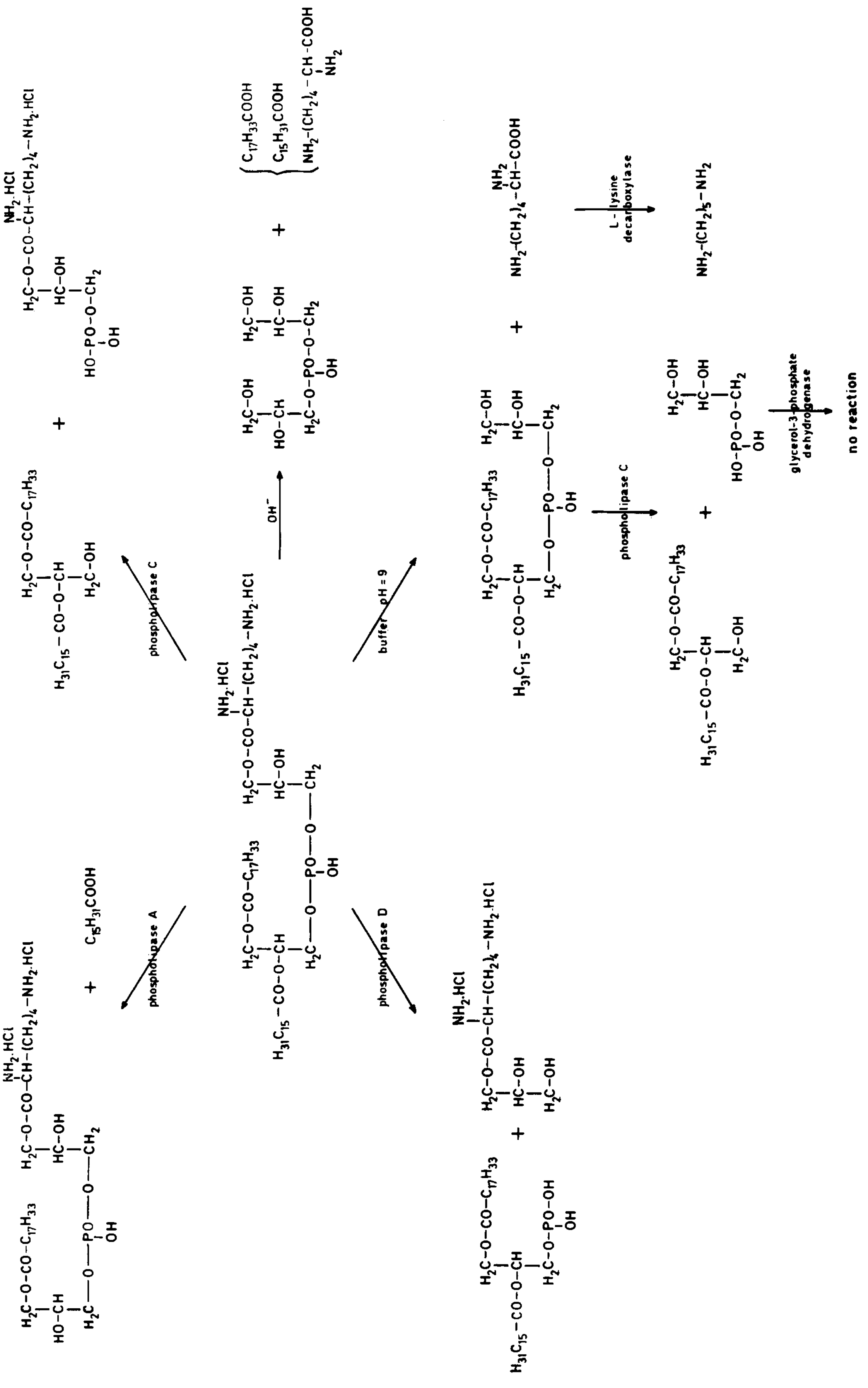
Infrared Spectra. The infrared spectrum of the synthetic lysyl ester of phosphatidylglycerol (Figure 1) closely resembles that of the natural compound. Small differences are observed with the spectrum of the alanine ester (Bonsen *et al.*, 1965; Baer and Jagannadha Rao, 1966) owing to the different number of amino groups.

Alkaline Hydrolysis (Scheme II). Alkaline hydrolysis of both compounds according to Dawson *et al.* (1960, 1962) yielded glycerylphosphorylglycerol as the only water-soluble product, as was demonstrated on paper chromatograms (Whatman No. 1) with propanol–concentrated ammonia–water (6:3:1, v/v), with identical R_F value (0.67) as a sample of glycerylphosphorylglycerol, obtained by alkaline hydrolysis of synthetic phosphatidylglycerol (Bonsen *et al.*, 1966). Since no lysine was observed on the paper chromatograms, this amino acid was apparently adsorbed by the strongly acidic Dowex resin.

Houtsmuller and van Deenen (1965) incubated ultrasonically emulsified phosphatidylglycerol in a sodium borate buffer (pH 9.0) for 30 min and found a practically complete hydrolysis into free lysine and 3-phosphatidyl-1′-glycerol. Treatment of 50 mg of the synthetic product in 25 ml of ether with an aqueous sodium borate buffer (0.05 M, pH 9.0) for 4 hr also gave identical behavior, indicating qualitatively a similar reactivity of the lysyl group.

The free amino acid in the water layer was incubated with L-lysine decarboxylase (EC 4.1.1.18) (for experimental details, see Houtsmuller and van Deenen, 1965). A nearly complete conversion (>95%) to cadaverine confirmed the stereochemical nature of the amino acid, indicating that little or none of the lysine has been racemized during the synthesis. Phosphatidylglycerol, resulting from the mild alkaline treatment of the lysyl ester, was subsequently degraded with phospholipase C from *Bacillus cereus* (EC 3.1.4.3) (Haverkate and van Deenen, 1964, 1965). This reaction furnished quantitatively a diglyceride, which had the same R_F value on thin layer chromatograms as that of synthetic 1,2-distearoylglycerol, and glycerol phosphate, which showed an R_F value (0.32) identical with that of racemic glycerol 1-phosphate on paper chromatograms with propanol–concentrated ammonia–water (6:3:1, v/v) as solvent system. This glycerol phosphate resisted completely the action of glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) (according to the method of Bublitz and Kennedy, 1954), indicating that the terminal glycerol moiety has the same stereochemical configuration as that found in the natural lysyl ester of phosphatidylglycerol (Houtsmuller and van Deenen, 1965) and in phosphatidylglycerol from spinach leaves and *B. cereus* (Haverkate and van Deenen, 1964, 1965).

SCHEME II: Chemical and Enzymic Degradation of 1-Oleoyl-2-palmitoylglycerol-3-phosphoryl-1′-(3′-O-L-lysyl)glycerol.



Enzymic Hydrolysis (*Scheme II*)

Phospholipase A. Hydrolysis of 10 mg of the synthetic lysyl ester of phosphatidylglycerol in 1 ml of ether with 2 mg of phospholipase A (EC 3.1.1.4) from *Crotalus adamanteus* in 1 ml of a collidine buffer (0.1 M, pH 6.5, with 5×10^{-3} M $CaCl_2$) gave in 3 hr a 100% breakdown into a lyso compound, containing more than 99% oleic acid. The released fatty acid was exclusively palmitic acid. Under these conditions no hydrolysis of lysine was observed. The natural compound was also completely hydrolyzed, under the same conditions, by the stereochemically specific phospholipase A (van Deenen and de Haas, 1963), indicating that the phosphatidyl moieties of the synthetic and natural lysyl ester of phosphatidylglycerol exhibit the same stereochemical configuration.

Phospholipase C. Incubation of the natural lysyl ester with phospholipase C in Tris buffer (pH 7.0) in the presence of ether showed the formation of a diglyceride and a water-soluble product, tentatively identified as lysylglycerol phosphate. Some nonenzymic release of the amino acid, however, was also observed (Houtsmuller and van Deenen, 1965). The synthetic product (10 mg), treated with phospholipase C (EC 3.1.4.3) from *B. cereus* (0.5 mg of protein) in Tris buffer (1 ml, 0.1 M, pH 6.6), underwent complete hydrolysis into a diglyceride, with R_F value identical with that of synthetic 1,2-distearoylglycerol. The water layer from the enzymic hydrolysis was extracted with ether and then treated with an equal volume of 20% trichloroacetic acid. The precipitate (denaturated enzyme) was removed and the filtrate was evaporated *in vacuo*. The residue was dissolved in a small volume of water and four volumes of ethanol were added. A precipitate was formed after cooling at −15°. This product was subjected to high-voltage paper electrophoresis (50 v/cm) with a pyridine–acetic acid–water buffer (pH 3.5) for 1 hr. A spot could be detected which contained an amino group (positive ninhydrin reaction) and phosphorus (positive reaction with the molybdate reagent), and which had the same migration distance (7.5 cm) as a synthetic reference of 3-*O*-L-lysylglycerol 1-phosphate.

Phospholipase D. Houtsmuller and van Deenen (1965) could not obtain hydrolysis of their isolated lysylphosphatidylglycerol with a commercial preparation of phospholipase D. A freshly prepared phospholipase D preparation (EC 3.1.4.4) from savoy cabbage or Brussel sprouts, according to Davidson and Long (1958), however, was found to hydrolyze the synthetic and natural compound. In both cases the phosphorus-containing hydrolysis product possessed the same chromatographic properties as synthetic phosphatidic acid (Bonsen and de Haas, 1967). The phosphatidic acid formed was removed by repeated extraction with ether. The water layer was treated with an equal volume of 20% trichloroacetic acid. The heavy precipitate was centrifuged. The excess of trichloroacetic acid was extracted with ether. The resulting aqueous solution was subjected to high-voltage paper electrophoresis (for details, see above). In both enzymic degradations lysylglycerol could be detected, which gave a positive reaction with the ninhydrin reagent and periodate–Schiff reagent, and with the same migration distance (16 cm) as synthetic 1-*O*-L-lysylglycerol.

Apart from differences in fatty acid composition, no differences in chemical and physical properties were detected between synthetic 3-phosphatidyl-1′-(3′-*O*-L-lysyl)glycerol and the lysyl ester of phosphatidyl glycerol isolated from *S. aureus*. The results obtained by means of enzymatic hydrolyses established also that the stereochemical configuration of both compounds is identical.

Although the properties of the water-soluble products obtained after hydrolysis with phospholipase D indicated that the lysine is esterified to the primary hydroxyl group, this does not necessarily mean that the amino acid is attached at the same position in the phospholipid occurring in the bacterium. Further studies with the aid of fresh new model substances will be required to give unequivocal proof of the position of the linkage between phosphatidylglycerol and the amino acid. Other possibilities for the elucidation of this problem are given by the elegant work of Lennarz *et al.* (1966), who demonstrated that the enzymic formation of *O*-L-lysylphosphatidylglycerol in *S. aureus* involves a reaction between lysyl-sRNA and phosphatidylglycerol. Preliminary experiments by Dr. W. J. Lennarz (personal communication) with the aid of synthetic isomeric deoxyphosphatidylglycerols and related substances, prepared in this laboratory, suggested that the free primary hydroxyl group of phosphatidylglycerol acts as acceptor in the enzymic incorporation of lysine.

Experimental Section

Materials and General Methods. 2,3-Isopropylideneglycerol (I) was synthesized as described previously (Bonsen *et al.*, 1966). The silver salt of 1-oleoyl-2-palmitoylglycerol 3-(*O*-benzyl)phosphate (XI) was prepared as described in earlier publications (de Haas and van Deenen, 1961, 1963). 1,2-Distearoylglycerol was synthesized starting from 3-*O*-benzylglycerol, acylation with stearoyl chloride, and catalytic hydrogenolysis. The synthesis of phosphatidylglycerol and phosphatidic acid was described by Bonsen *et al.* (1966, 1967). 3-Lysylglycerol was obtained by reaction of glycerol 3-iodohydrin with di-*N*-*t*-butyoxycarbonyllysine, followed by treatment with trifluoroacetic acid. 3-Lysylglycerol 1-phosphate was prepared by phosphorylation of X with silver dibenzyl phosphate. The blocking groups were removed by catalytic hydrogenolysis and treatment with hydrogen chloride as described for the preparation of XIV. Microanalyses were carried out in the Analytical Department of the Laboratory of Organic Chemistry, University of Groningen. Melting points were determined on a Kofler hot plate and are uncorrected. Optical rotations were measured in a Lichtelectrisches Präzisions polarimeter 0.005°, Carl Zeiss. Infrared spectra were recorded with a Beckman IR-8 infrared spectrophotometer. The purity of intermediates and end products was checked by paper chromatography and by thin layer chromatography on silica gel as described previously (Daemen *et al.*, 1963).

2,3-Isopropylidene-1-O-benzylglycerol (II). Introduction of a benzyl group in 2,3-isopropylideneglycerol (I) was effected as described for the racemic compound by Howe and Malkin (1951), $[\alpha]_{\mathrm{D}}^{20}$ $-19°$ (in substance).

1-O-Benzylglycerol (III). The isopropylidene group of the foregoing product (II) was hydrolyzed with 10% acetic acid according to Howe and Malkin (1951). Distillation gave pure 1-*O*-benzylglycerol, $[\alpha]_{\mathrm{D}}^{20}$ $-5.80°$ (in substance). Lands and Zschocke (1965) reported $[\alpha]_{\mathrm{D}}$ $-5.85°$ (in substance).

1-O-Benzyl-3-p-toluenesulfonylglycerol (IV). The tosyl group was introduced with *p*-toluenesulfonyl chloride in pyridine. Isolation and purification of the racemic compound was reported extensively by Slotboom *et al.* (1963), $[\alpha]_{\mathrm{D}}^{20}$ $-6.07°$ (*c* 14, chloroform).

Anal. Calcd for $C_{17}H_{20}O_5S$ (336.40): C, 60.99; H, 5.99; S, 9.53. Found: C, 60.5; H, 6.0; S, 9.4.

1-O-Benzyl-2-O-t-butyl-3-p-toluenesulfonylglycerol (V). The foregoing product was allowed to react with liquid isobutene and a catalytic amount of sulfuric acid in methylene chloride. The purification was carried out as described for the racemic compound (Bonsen *et al.*, 1965), $[\alpha]_{\mathrm{D}}^{20}$ $-6.17°$ (*c* 10, chloroform).

Anal. Calcd for $C_{21}H_{28}O_5S$ (392.50): C, 64.25; H, 7.19; S, 8.17. Found: C, 64.0; H, 7.1; S, 9.3.

1-O-Benzyl-2-O-t-butylglycerol 3-Iodohydrin (VI). The *p*-toluenesulfonyl group in V was substituted by iodine by means of a threefold excess of sodium iodide in anhydrous acetone. After removal of the precipitated sodium *p*-toluenesulfonate the solution was evaporated to dryness *in vacuo*. The residue was dissolved in dry ether and filtered. The solvent was removed *in vacuo* and the remaining residue was chromatographed in the dark on a silica column with benzene–ether mixtures as eluents, to give pure VI as a colorless oil in a yield of 70%, $[\alpha]_{\mathrm{D}}^{20}$ $-3.92°$ (*c* 12, chloroform).

Anal. Calcd for $C_{14}H_{21}IO_2$ (348.21): C, 48.29; H, 6.08; I, 36.45. Found: C, 48.3; H, 6.0; I, 36.4.

Di-N-t-butoxycarbonyl-L-lysine. L-Lysine (18.25 g) (dihydrochloric acid salt) was dissolved in 200 ml of water, and 25 g of powdered magnesium oxide was added, followed by a solution of 57 g of *t*-butoxycarbonyl azide in 200 ml of dioxane. The mixture was stirred at 45° for 48 hr. After centrifugation of the excess of magnesium oxide, the same volume of ethyl acetate was added to the clear solution and allowed to stir with 300 g of Dowex 50W-X8 for 2 hr. The upper layer, containing the desired product in the acid form, was collected, dried over sodium sulfate, and evaporated *in vacuo*. The slightly yellowish oily residue could not be obtained in a crystalline form. Extensive drying over KOH at 0.001 mm resulted in a glasslike very hygroscopic material (60%), which could be powdered, with melting traject 35–40°. From this material a crystalline dicyclohexylammonium salt was prepared, mp 134.5–136°, $[\alpha]_{\mathrm{D}}^{20}$ $+5.89°$ (*c* 8, chloroform).

Anal. Calcd for $C_{28}H_{53}N_3O_6$ (527.73): C, 63.72; H, 10.12; N, 7.96. Found: C, 63.7; H, 10.1; N, 7.8.

1-O-Benzyl-2-O-t-butyl-3-(di-N-t-butoxycarbonyl)-L-lysylglycerol (VII). A mixture of 8.77 g of di-*N*-*t*-butoxycarbonyl-L-lysine, 2.56 g of anhydrous triethylamine, and 4.24 g of the iodo compound VI was heated at 105° for 4 hr. After cooling, the mixture was dissolved in ether and extracted with an aqueous sodium bicarbonate solution and with water. After drying over sodium sulfate, the solvent was evaporated *in vacuo*. The residue was applied to a silica column and eluted with benzene–ether mixtures. Compound VII was obtained as a colorless oil in a yield of 81%, $[\alpha]_{\mathrm{D}}^{20}$ $-4.75°$ (*c* 5, chloroform).

Anal. Calcd for $C_{30}H_{50}N_2O_8$ (566.72): C, 63.58; H, 8.89; N, 4.94. Found: C, 64.0; H, 8.8; N, 5.0.

2-O-t-Butyl-3-(di-N-t-butoxycarbonyl)-L-lysylglycerol (VIII). The foregoing product (VII) (5.54 g) was subjected to hydrogenolysis in absolute ethanol with palladium as a catalyst. A small amount of VII was removed by means of chromatography over silica with benzene–ether mixtures as eluents, to give 4.25 g of VIII (91%) as a colorless oil, $[\alpha]_{\mathrm{D}}^{20}$ $+7.65°$ (*c* 7, chloroform).

Anal. Calcd for $C_{23}H_{44}N_2O_8$ (476.60): C, 57.95; H, 9.30; N, 5.88. Found: C, 57.9; H, 9.4; N, 5.9.

1-O-p-Toluenesulfonyl-2-O-t-butyl-3-(di-N-t-butoxycarbonyl)-L-lysylglycerol (IX). Introduction of a tosyl group in VIII was effected with *p*-toluenesulfonyl chloride in pyridine. The mixture was allowed to stir for 3 days at room temperature. After cooling to 0°, the excess of *p*-toluenesulfonyl chloride was destroyed with 5 ml of water. This mixture was taken up in ether and subsequently washed with ice-cold 0.5 N sulfuric acid, sodium bicarbonate solution, and water. The solution was dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was chromatographed over silica with benzene–ether mixtures as eluents to give IX as a colorless oil in a yield of 80%, $[\alpha]_{\mathrm{D}}^{20}$ $-7.67°$ (*c* 5, chloroform).

Anal. Calcd for $C_{30}H_{50}N_2O_{10}S$ (630.79): C, 57.12; H, 7.99; N, 4.44; S, 5.08. Found: C, 57.2; H, 8.0; N, 4.5; S, 4.8.

3-(Di-N-t-butoxycarbonyl)-L-lysyl-2-O-t-butylglycerol 1-Iodohydrin (X). The substitution of the tosyl group in IX by iodine was carried out by heating at reflux with a twofold excess of sodium iodide in dry acetone for 24 hr. Sodium *p*-toluenesulfate was collected by filtration and the filtrate was evaporated *in vacuo*. The residue was extracted with ether and the ethereal solution was washed with a 5% sodium thiosulfate solution and water. After drying over sodium sulfate the solvent was removed *in vacuo* and the residue was applied to a silica column. Benzene–ether mixtures were used as eluents. Compound X was obtained as a colorless viscous oil in a yield of 95%, $[\alpha]_{\mathrm{D}}^{20}$ $-2.30°$ (*c* 8, chloroform).

Anal. Calcd for $C_{23}H_{43}IN_2O_7$ (586.49): C, 47.10; H, 7.39; I, 21.63; N, 4.78. Found: C, 47.5; H, 7.5; I, 21.2; N, 5.0.

*1-Oleoyl-3-palmitoylglycerol-3-(O-benzyl)phosphoryl-1′-(3′-(di-N-t-butoxycarbonyl)-*L*-lysyl-2′-O-t-butyl)-glycerol (XII).* The iodo derivative X (3.63 g) was allowed to react with 5.5 g (20% excess) of the silver salt of 1-oleoyl-2-palmitoylglycerol 3-(*O*-benzyl)phosphate (XI) in anhydrous toluene (freshly distilled over $LiAlH_4$) at 110° for 1.5 hr. The precipitate of silver iodide was removed by centrifugation and the supernatant was evaporated *in vacuo.* The residue was dissolved in pentane and washed with a sodium bicarbonate solution and water. After drying over sodium sulfate, the solvent was removed *in vacuo.* The residue was triturated with acetone to remove the excess of benzylphosphatidic acid. The remaining solid was discarded, and the solution was evaporated *in vacuo* and chromatographed on silica with benzene–ether mixtures. All fractions still contained small amounts of benzylphosphatidic acid which could be removed by a rapid elution of the triester over an alumina column. XII was obtained as a colorless viscous oil in a yield of 55%, $[\alpha]_D^{20}$ +0.80° (*c* 8, chloroform).

Anal. Calcd for $C_{67}H_{119}N_2O_{15}P$ (1233.61): C, 66.04; H, 9.72; N, 2.27; P, 2.51. Found: C, 65.6; H, 9.8; N, 2.5; P, 2.5.

*Sodium Salt of 1-Oleoyl-2-palmitoylglycerol-3-phosphoryl-1′-(3′-(di-N-t-butoxycarbonyl)-*L*-lysyl-2′-O-t-butyl)glycerol (XIII).* The phosphotriester XII was debenzylated with barium iodide in anhydrous acetone at boiling temperature for 3 hr. No precipitate of the barium salt of XIII was formed. The solution was, therefore, evaporated to dryness *in vacuo*, and the residue was extracted with ether. The ethereal solution was washed with a sodium sulfate solution to convert the barium salt of XIII into the sodium salt and dried over sodium sulfate. After removal of the solvent, the residual oil was applied to a silica column and eluted with chloroform. The pure fractions were again converted into the sodium salt to give 70% of XIII as a colorless waxy solid, $[\alpha]_D^{20}$ +1.02° (*c* 6, chloroform).

Anal. Calcd for $C_{60}H_{112}N_2NaO_{15}P$ (1155.49): C, 62.36; H, 9.77; N, 2.42. Found: C, 62.6; H, 10.0; N, 2.3.

*1-Oleoyl-2-palmitoylglycerol-3-phosphoryl-1′-(3′-*L*-lysyl)glycerol (XIV) (as Dihydrochloric Acid Salt).* The pure sodium salt XIII was dissolved in anhydrous chloroform (freshly distilled over P_2O_5) and at 0° a stream of dry hydrogen chloride was allowed to pass through the solution for 1.5 hr. The solution was evaporated *in vacuo* at 0°. The solid material was recrystallized from chloroform–acetone at −15° to give a white crystalline hygroscopic product in a yield of 91%, mp 199–201°, $[\alpha]_D^{20}$ +4.1° (*c* 8, chloroform).

Anal. Calcd for $C_{46}H_{91}Cl_2N_2O_{11}P \cdot H_2O$ (968.11): C, 57.06; H, 9.68; Cl, 7.32; N, 2.89; P, 3.20. Found: C, 56.9; H, 9.6; Cl, 7.3; N, 3.1; P, 3.0.

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