CHEMISTRY OF LIPIDES

COMMUNICATION 8.* SYNTHESIS OF S-α-L-PHOSPHATIDYL-γ-L-ALANYLGLYCEROL

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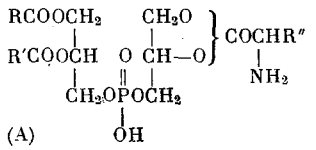
In recent years it was established by mean of studies carried out in several laboratories that the phospholipides of certain bacteria contain alanine, glycine, lysine and other amino acids in the bound form [3-7]. The theories were expressed that the lipoamino acid complexes can take part in the synthesis of protein both in microorganisms, as well as in the higher plants and animals [8-11].

On the basis of the analytical and spectral data it was proposed that the bacterial amino acids have the structure of the amino acid esters of phosphatidylglycerol (A) [3-5, 7], in which connection the position of the amino acid moiety remained unestablished.



(A)

Recently two papers were published on the synthesis of compounds of the (A) type [12, 13]. Since both of the syntheses lead to compounds that are either partially or completely racemic, the structure of the natural lipoamino acids has still not been conclusively ascertained.

As regards the configuration of the lipoamino acids, then for the lysine ester [A, $R'' = (CH_2)_4NH_2$], isolated from Staphylococcus aureus, on the basis of the enzymatic cleavage results it was concluded that the glycerophosphate moiety, bearing the aliphatic acids, has the L-configuration, while the glycerophosphate moiety, esterified with lysine, has the D-configuration [7]. It seems probable that the other lipoamino acids also have the same configuration.

In an attempt to solve the problem of the structure of the lipoamino acids by comparing them directly with the synthetic compounds, we synthesized α-L-phosphatidyl-γ-L-alanylglycerol (IX), with an S-configuration of the glycerol moiety attached to the amino acid.† The route for the synthesis of this compound (see scheme)‡ was based on the condensation of the silver salt of benzyl-(1,2-distearoylglyceryl)-phosphoric acid (VII)** with iodide (VI). The first of these compounds was synthesized by the condensation of glycerol 1,2-distearoyl-3-iodohydrin with silver dibenzyl phosphate by the method used to obtain the analogous racemate [17]. To obtain iodide (VI) we used the general principles previously developed by us for the synthesis of optically active diglycerides, described in [18]. The methyl ester of R-1-O-trityl-3-glyceric acid (I), described in this paper, was benzylated and then reduced with aluminum lithium hydride to S-1-O-trityl-2-O-benzylglycerol (III), which was then esterified with N-carbobenzoxy-L-alanine by the carbodiimide method to R-1-O-trityl-2-O-benzyl-3-(N-carbobenzoxy-L-alanyl)glycerol (IV). The latter compound was also synthesized from the readily available D(S)-1,2-O-isopropylideneglycerol (X) [19] by

*See [1, 2] for communications 6 and 7.

†See [14] for preliminary communication.

‡St = stearoyl; the abbreviations of the amino acid moieties and the protective groups are given in harmony with the Rules of the Joint IUPAC–IUB Commission [15].

**See [16] for the numbering of the C atoms in the optically active derivatives of glycerol.

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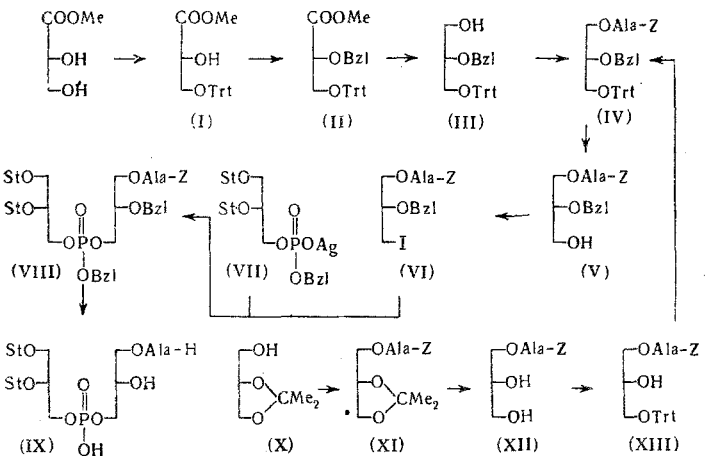
the esterification of (X) with N-carbobenzoxy-L-alanine, removal of the isopropylidene grouping by mild acid hydrolysis, and successive tritylation and benzylation (X → XI → XII → XIII → IV, see scheme).

Scheme



The selective cleavage of the trityl protection from (IV) was achieved by chromatographing (IV) on active silica gel [20, 21], or by hydrolysis with dilute hydrochloric acid under conditions sufficiently mild to prevent acyl migration. The formed R-2-O-benzyl-3-(N-carbobenzoxy-L-alanyl)glycerol (V) was converted via the corresponding tosylate to iodide (VI).

The condensation of iodide (VI) with the silver salt (VII) gave the phosphatidylglycerol derivative (VIII). All three protective groups were removed from this compound by hydrogenolysis over palladium. The hydrogenolysis product – S-1-(1',2'-distearoylglycerylphosphoryl)-3-L-alanylglycerol (IX) was obtained as a colorless microcrystalline powder with a vague melting point and a positive specific rotation. A comparison of the synthesized lipoamino acid (IX) with the natural product will be described in the next communication.

EXPERIMENTAL

The melting points were determined in a Kofler block and are corrected. The angles of rotation were measured on a "Hilger" polarimeter in dioxane (except the specially mentioned cases) with an accuracy of ±1-2°. The thin-layer chromatography (TLC) was run on KSK silica gel (fraction smaller than 150 mesh) in a bound gypsum layer or on aluminum oxide in a loose layer. For detecting the spots we used: A) spraying with concentrated H_2SO_4; B) spraying with concentrated H_2SO_4 and subsequent heating; C) spraying with an 0.05% methanol solution of morin and subsequent ultraviolet irradiation. For the column chromatography we used: commercial silicic acid (fraction smaller than 150 mesh), KSK silica gel (fraction smaller than 150 mesh), washed free of traces of metal using 10% HNO_3 solution and dried at 120°, and aluminum oxide (III activity).

Methyl Ester of R-1-O-Trityl-3-glyceric Acid (I). Using the method described for the synthesis of the corresponding racemate [22], and from 37 g of D(R)-1,2-O-isopropylideneglyceraldehyde [19] we obtained 10.7 g (36%) of crude methyl ester of the R-3-glyceric acid as a pale yellow oil. A small amount of the latter was distilled at 140° (bath) (15 mm); here a colorless oil with d_4^{20} 1.2795, n_D^{20} 1.4468, and $[\alpha]_D$ −4.7° (neat) was obtained in low yield (a large amount of polymeric still residue was obtained). Literature data [23]: bp 119-120° (14 mm); d_{15}^{15} 1.2798; $[\alpha]_D^{15}$ −4.8°.

With stirring, 37.5 g of triphenylchloromethane was added to a solution of 10.7 g of crude methyl glycerate in 150 ml of absolute pyridine, and the mixture was stirred for 40 min and allowed to stand at room temperature for 2 days. The mixture was evaporated in vacuo to half volume, 300 ml of ether was added to the residue, and the obtained precipitate of pyridine hydrochloride was separated and washed with ether (2 ×50 ml). The combined filtrates were washed in succession with chilled (to 0°) 5% HCl solution, water, saturated aqueous $NaHCO_3$ solution, water and brine solution, and dried over Na_2SO_4. Then the

drying agent was filtered, washed with ether, and the solvent was removed in vacuo to give 39.3 g of residue, which based on the data of TLC on silica gel (system petroleum ether–ether, 2:1; developer A) is a mixture of (I) and triphenylcarbinol. A part of the obtained mixture (1.42 g) was chromatographed on a column containing 100 g of KSK silica gel, following the composition of the fractions by TLC. 0.52 g of triphenylcarbinol was eluted with benzene containing 5% chloroform, and 0.72 g (54.5%) of (I) was eluted with ether as an oil that crystallized on standing. After 2 recrystallizations from a mixture of ether and hexane, mp 127-128°; $[\alpha]_D^{18}$ −5.3° (C 5.7). Found %: C 76.36; H 6.22. $C_{23}H_{22}O_4$. Calculated %: C 76.22; H 6.12.

S-1-O-Trityl-2-O-benzylglycerol (III). To a solution of 1 g of (I) and 1.2 ml of benzyl bromide in 50 ml of absolute benzene was added 4.6 g of dry, freshly prepared silver oxide. The mixture was stirred for 6 h, and an additional 0.7 ml of benzyl bromide and 1 g of silver oxide were added, and the stirring was continued for another 6 h. The mixture was filtered, the precipitate was washed with ethyl acetate, and the combined filtrates were evaporated. The oily residue of the crude methyl ester of 1-O-trityl-2-O-benzyl-3-glyceric acid (II) was dissolved in 15 ml of ether and added in 10 min to a suspension of 380 mg of lithium aluminum hydride in 50 ml of ether. The mixture was refluxed for 30 min, cooled, 2 ml of ethyl acetate was added in drops, the mixture was stirred for 2 min, and then, with stirring, were added 50 ml of ether, 6.5 ml of saturated aqueous $(NH_4)_2SO_4$ solution and dry $(NH_4)_2SO_4$ until a filterable precipitate was obtained. The mixture was filtered, the precipitate was washed well with ether (2×50 ml) and dioxane (50 ml), and the filtrate was dried over Na_2SO_4 and dried. The residue was chromatographed on 130 g of aluminum oxide in the system benzene–ethyl acetate (gradient elution), controlling the composition of the fractions by TLC on aluminum oxide (system benzene–ethyl acetate, 5:1; developer A). 0.85 g of colorless oil, which crystallized on standing, was eluted with benzene containing 15-20% of ethyl acetate. After recrystallization from benzene–hexane, mp 81-82°; $[\alpha]_D^{23}$ −13.2° (C 4.2). Found %: C 81.75; H 6.65. $C_{29}H_{28}O_3$. Calculated %: C 81.52; H 6.84.

R-1,2-O-Isopropylidene-3-(N-carbobenzoxy-L-alanyl)glycerol (XI). With cooling in ice and stirring, a solution of 10 g of dicyclohexylcarbodiimide in 40 ml of pyridine was added in 30 min to a solution of 5 g of 1,2-O-isopropylideneglycerol (X) and 8.8 g of N-carbobenzoxy-L-alanine in 80 ml of pyridine, after which the mixture was kept at 0° for 6 h and at room temperature for 3 days. The precipitate of dicyclohexylurea was suction-filtered, washed with ether, and the filtrate was evaporated in vacuo to a volume of 50 ml, diluted with 300 ml of benzene, and kept at 5° for 2 h. The precipitate was suction-filtered, washed with benzene, and the filtrate was evaporated in vacuo to give 16 g of crude (XI) as a brown viscous oil. A part of the latter (0.35 g) was dissolved in benzene and chromatographed on a column containing 35 g of KSK silica gel, using gradient elution with the system chloroform–mixture of chloroform and ethyl acetate (1:1) (control by TLC on silica gel; system benzene–ethyl acetate, 2:1; developer B). 140 mg of a mixture of dicyclohexylurea and unidentified substances was eluted with chloroform containing 0-5% ethyl acetate, and 180 mg of acetonide (XI) was eluted with chloroform containing 5-10% ethyl acetate as a very viscous oil, which distilled at 190° (bath) (0.1 mm); $[\alpha]_D^{18}$ −7.4° (C 6.8). Found %: C 60.65; H 6.75; N 4.17. $C_{17}H_{23}NO_6$. Calculated %: C 60.52; H 6.87; N 4.15.

R-3-(N-Carbobenzoxy-L-alanyl)glycerol (XII). The crude acetonide (XI) (13.5 g) was stirred for 4 h with 20 ml of 10% acetic acid solution at 60°, after which the solution was decanted, and the tarry deposit was treated again with 10% acetic acid solution and decanted. The combined solutions were evaporated, and the residue (9.5 g) was chromatographed on a column containing 1.5 kg of silicic acid (gradient elution with the system chloroform–methanol, control by TLC in the system chloroform–methanol, 9:1; developer B); 6.1 g of (XII) was eluted with chloroform containing 6-10% methanol as a pale yellow viscous oil [yield 64%, based on (X)]; $[\alpha]_D^{18}$ −13.5° (C 8.5). Infrared spectrum (as a film): 3400 (s, wide), 1720 (s, wide), 1534 (s), 1460 (med), 1263 (s) cm^{-1}. Found %: C 56.68; H 6.94; N 4.61. $C_{14}H_{19}NO_6$. Calculated %: C 56.56; H 6.44; N 4.71.

R-1-O-Trityl-3-(N-carbobenzoxy-L-alanyl)glycerol (XIII). 2.24 g of (XII) was tritylated under the conditions used to obtain (I). The reaction product was chromatographed on 300 g of silicic acid (control by TLC on silica gel: system chloroform–acetone, 10:1, developer B). The triphenylcarbinol was eluted with a 3:1 mixture of benzene and chloroform, and 2.6 g (64%) of (XIII) was eluted with chloroform containing 10% acetone as an oil, which crystallized on standing. After recrystallization from ether, mp 127-129°; $[\alpha]_D^{17}$ −12.2° (C 4.2). Found %: C 73.51; H 6.30; N 2.67. $C_{33}H_{33}NO_6$. Calculated %: C 73.45; H 6.16; N 2.60.

R-1-O-Trityl-2-O-benzyl-3-(N-carbobenzoxy-L-alanyl)glycerol (IV). A. With cooling in ice, a solution of 0.6 g of dicyclohexylcarbodiimide in 2 ml of pyridine was added in 20 min to a solution of 1 g of (III) and 0.6 g of N-carbobenzoxy-L-alanine in 6 ml of pyridine, after which the mixture was stirred for 3 h under cooling, and then 8 h at room temperature. The data of TLC on silica gel (system benzene–ether, 3:1; developer B) show that the mixture contains substantial amounts of unreacted (III). For this reason it was treated 3 times at 10-h intervals with portions composed of 0.2 g of N-carbobenzoxy-L-alanine and 0.2 g of dicyclohexylcarbodiimide. 8 h after adding the last portion the mixture was diluted with 50 ml of benzene, filtered, the precipitate washed with benzene, and the combined filtrates evaporated. The residue was chromatographed on 250 g of KSK silica gel, using gradient elution with the system benzene–benzene containing 50% ethyl acetate. 890 mg (60%) of (IV) was eluted with benzene containing 5-10% ethyl acetate as an oil with $[\alpha]_D^{22}$ −15.8° (C 5.5). Infrared spectrum (as a film): 3340 (s), 1726 (s), 1747 (shoulder), 1531 (s) cm^{-1}. Found %: C 76.48; H 6.03; N 2.14. $C_{40}H_{39}NO_6$. Calculated %: C 76.29; H 6.24; N 2.22.

B. 0.74 g of (XIII) was benzylated as described above [see the preparation of (III)]. After separating and washing the precipitate, the filtrate was evaporated in vacuo, and the residue was chromatographed on a column containing 120 g of KSK silica gel (control by TLC on silica gel; system benzene–ether, 2:1; developer B). The benzyl bromide and other nonpolar impurities were eluted with benzene containing 0 to 5% ether, while 800 mg (92% yield) of (IV) was eluted with a mixture of benzene containing 10-15% ether as an oil with $[\alpha]_D^{20}$ −14.1° (C 5.6). Based on the chromatographic behavior and infrared spectra, the samples of (IV) obtained by methods A and B were identical.

R-2-O-Benzyl-3-(N-carbobenzoxy-L-alanyl)glycerol (V). A. A solution of 800 mg of (IV) in 20 ml of benzene was transferred to a column containing 150 g of silica gel [24], activated at 150°, and the column was developed with 150 ml of benzene and allowed to stand at room temperature for 10 h. Then the elution was started, controlling the composition of the eluates by TLC (system benzene–ethyl acetate, 3:1; developer B). The triphenylcarbinol was eluted with benzene, 150 mg of unreacted (IV) was eluted with a mixture of benzene containing 5-10% ether, and then 303 mg of (V) was eluted with a 3:7 to 2:8 benzene–ether mixture as a viscous oil; $[\alpha]_D^{22}$ −12.5° (C 6.8). Found %: N 3.43. $C_{21}H_{25}NO_6$. Calculated %: N 3.62.

B. To a solution of 350 mg of (IV) in 25 ml of dioxane was added 0.7 ml of 10% HCl solution. The mixture was kept at room temperature for 40 h, diluted with 50 ml of ether, and the solution was washed with cold aqueous $NaHCO_3$ solution, water and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed (see method A) to give 155 mg of (V); $[\alpha]_D^{22}$ −12.1° (C 2.8).

Glycerol S-2-O-Benzyl-3-(N-carbobenzoxy-L-alanyl)-1-iodohydrin (VI). With stirring, a solution of 130 mg of (V) in 0.5 ml of ether was added to a solution of 150 mg of p-toluenesulfonyl chloride in 0.5 ml of pyridine at 0°. The mixture was kept at 18-20° for 15 h and then at 30° for another 7 h, after which it was diluted with 20 ml of benzene, washed twice with saturated aqueous $NaHCO_3$ solution, then with water, 2% HCl solution, again with water, then with brine, dried over Na_2SO_4, and evaporated in vacuo. The oily residue was dissolved in 4 ml of absolute acetone, 250 mg of dry NaI was added, and the solution was kept in a sealed glass ampule in the dark for 5 h at 80-85°. To the cooled mixture, transferred to a separatory funnel, was added 30 ml of benzene, and the mixture was washed in succession with 1% aqueous $Na_2S_2O_3$ solution, water and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was dissolved in benzene and filtered through a column containing 10 g of KSK silica gel (the column was rinsed with 30 ml of benzene containing 20% ethyl acetate). Evaporation of the eluate gave 150 mg (90% yield) of (VI) as a yellowish oil with $[\alpha]_D^{24}$ −14.4° (C 4.4; benzene). Infrared spectrum (as a film): 3380 (s, wide), 1730 (v.s, wide), 1530 (s), 1456 (s), 740 (s), 692 (s). Found %: C 51.24; H 5.06; I 25.18; N 2.63. $C_{21}H_{24}INO_5$. Calculated %: C 50.70; H 4.86; I 25.52; N 2.63.

Silver Benzyl-(1,2-distearoylglyceryl)phosphate (VII). Using the method described in [17], from 2.15 g of glycerol 1,2-distearoyl-3-iodohydrin [25] and 1.3 g of silver dibenzyl phosphate we obtained dibenzyl (1,2-distearoylglyceryl) phosphate in 85% yield; mp 57-58° (from ether–hexane); $[\alpha]_D^{17}$ +2.4° (C 10), +2.3° (C 10, $CHCl_3$). Found %: C 71.65; H 10.15; P 3.43. $C_{53}H_{89}O_8P$. Calculated %: C 71.91; H 10.14; P 3.51.

From the latter was obtained [25] the silver salt (VII) as a colorless powder. After recrystallization from chloroform–acetone, mp 96-99° (decomp.) (softens at 85°); $[\alpha]_D^{24}$ +3.8° (C 4, $CHCl_3$).

S-1-[(1',2'-Distearoylglyceryl)benzylphosphoryl]-2-O-benzyl-3-(N-carbobenzoxy-L-alanyl)glycerol (VIII). A mixture of 110 mg of (VI), 200 mg of the silver salt (VII) and 5 ml of benzene was refluxed for 5 h, with vigorous stirring, in the dark, in an argon atmosphere. The AgI precipitate was suction-filtered, washed with ether, the combined filtrates were evaporated in vacuo, and the residue was chromatographed on a column containing 40 g of silica gel (control by TLC; system benzene–ethyl acetate, 4:1; developer C). A small amount of unreacted (VI) was eluted with benzene containing 0-5% ethyl acetate, and 120 mg (47%) of (VIII) was eluted with a mixture of benzene containing 10 to 15% ethyl acetate as an oil, which gradually congealed to a waxy mass. After recrystallization from ether–methanol at −5°, mp 38-40°; $[\alpha]_D^{20}$ −2.9° (C 10). Found %: C 69.04; H 9.08; N 1.43; P 2.34. $C_{67}H_{106}NO_{13}P$. Calculated %: C 69.10; H 9.18; N 1.20; P 2.66.

S-1-(1',2'-Distearoylglycerylphosphoryl)-3-L-alanylglycerol (IX). A. 180 mg of triphosphate (VIII) in 10 ml of dioxane was hydrogenated for 12 h over 200 mg of previously reduced 5% PdO/$BaSO_4$ [26]. The catalyst was suction-filtered, washed with a 2:1 mixture of chloroform and methanol, and the filtrate was evaporated. The residue, based on the data of TLC on silica gel (system chloroform–methanol–water, 65:25:3; developers – C and ninhydrin), is devoid of the starting (VIII) and contains two ninhydrin-positive substances with R_f 0.56 and 0.37. The mixture was chromatographed on a column containing 25 g of KSK silica gel, and the first substance (IX, 70 mg) was eluted with a mixture of chloroform containing 10-15% methanol as a colorless powder. After recrystallization from chloroform–acetone, mp 70-75° (softens at 55-57°); $[\alpha]_D^{24}$ +6.8° (C 6.3, $CHCl_3$). The substance does not depress the melting point when mixed with a sample of the material obtained by method B (see below). Infrared spectrum (KBr tablet): 3400 (s, wide), 2930 (s), 2855 (s), 1750 (s), 1473 (med), 1388 (med), 1243 (s, wide), 1116 (med), 1078 (med), 965 (med) cm^{-1}.

35 mg of unidentified substance was eluted with a mixture of chloroform containing 20% methanol as a colorless powder, which after recrystallization from chloroform–acetone had mp ~115° (softens at ~85°); $[\alpha]_D^{24}$ +7.4° (C 2.3, $CHCl_3$). Infrared spectrum (KBr tablet): 3420 (s, wide), 2930 (s), 2855 (s), 1748 (s), 1470 (med), 1220 (s, wide), 1110 (s), 1070 (s), 972 (med), 850 (med) cm^{-1}. Found %: C 61.81; H 10.39; N 1.42; P 4.31. Based on the TLC data, the amount of (IX) in the mixture decreases when the time of hydrogenation is increased, while the amount of the more polar substance increases.

B. 90 mg of (VIII) in 30 ml of ethyl acetate was hydrogenated over 30 mg of palladium black in the presence of 0.2 ml of glacial acetic acid for 4 h. Based on the TLC data (see above), the obtained mixture contains, among other substances, one ninhydrin-positive component with R_f 0.56. It was isolated by chromatographing (see method A), yield 30 mg, mp 70-75° (softens at ~55°); $[\alpha]_D^{18}$ +7.8° (C 1.5, $CHCl_3$). Found %: C 63.90; H 10.30; N 1.58; P 3.79. $C_{45}H_{88}NO_{11}P$. Calculated %: C 63.57; H 10.43; N 1.65; P 3.65.

CONCLUSIONS

The total synthesis of S-α-L-phosphatidyl-γ-L-alanylglycerol was accomplished, a lipoamino acid that is optically active at all of the asymmetric centers.

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