SYNTHESIS OF OPTICALLY ACTIVE LIPOAMINOACIDS

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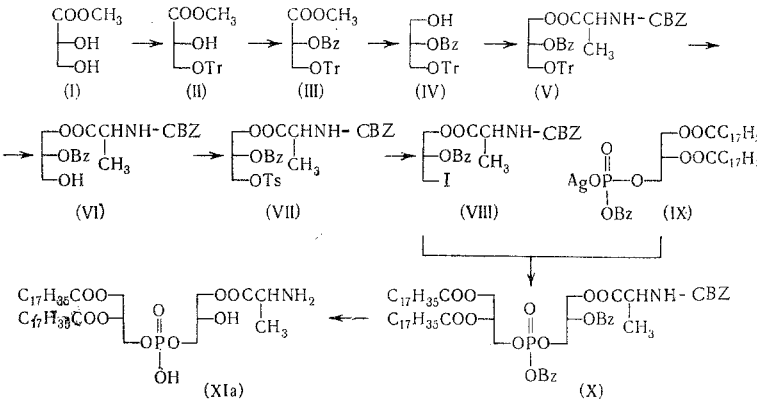
It has recently been shown that the phospholipids of some bacteria contain alanine, glycine, lysine, or ornithine in the bound form [1-4]. Since the hypothesis that lipoaminoacid complexes may participate in the synthesis of protein both in microorganisms and in higher plants and animals had been expressed even before this [5-8], the discovery of lipoaminoacids caused considerable interest. On the basis of analytical and spectroscopic data for the bacterial lipoaminoacids, the structure of aminoacid esters of the phosphatidylglycerol type (XI) has been proposed [1].

Syntheses of completely or partially racemic materials of type (XI) have been carried out [9, 10], but the question of the position of the aminoacid residue and of the absolute configuration of the natural lipoaminoacids still remains open. To investigate this question, we have carried out the synthesis of the two possible isomers of α-L-distearoylphosphatidyl-γ-L-alanylglycerol (XIa) and (XIb), which differ by the configuration of the glycerol residue esterified with the alanine.

EXPERIMENTAL

The synthesis of the epimer (XIa) was carried out in the following way (scheme 1)

Scheme 1



Bz = $C_6H_5CH_2$; Tr = $(C_6H_5)_3C$; Ts = n-$CH_3C_6H_4SO_2$; CBZ = $C_6H_5CH_2OCO$

Benzylation of methyl D-β-tritylglycerate (II) — m.p. 127-128° (from ether—hexane), $[\alpha]_D^{18}$ — 5.3° (c 5.7, dioxane) — by means of benzyl bromide in the presence of Ag_2O in benzene gave methyl R-α-benzyl-β-tritylglycerate (III) which, without purification, was reduced with $LiAlH_4$ to S-β-benzyl-γ-tritylglycerol (IV); m.p. 81-82° (from benzene—hexane), $[\alpha]_D^{23}$ —13.2° (c 4.2, dioxane). Esterification of the latter with N-carbobenzoxy-L-alanine in the presence of dicyclohexylcarbodiimide gave the oily R-α-N-carbobenzoxy-L-alanyl-β-benzyl-γ-tritylglycerol (V); $[\alpha]_D^{22}$ —15.8° (c 5.5, dioxane). Elimination of the trityl group from (V) by acid hydrolysis on silica gel [11, 12] or by dilute HCl in dioxane gave R-α-N-carbobenzoxy-L-alanyl-β-benzylglycerol (VI) (viscous oil); $[\alpha]_D^{22}$ 12.5° (c 6.8, dioxane).

The tosylation of (VI) led to R-α-N-carbobenzoxy-L-alanyl-β-benzyl-γ-tosylglycerol (VII). Without purification, the latter was converted by boiling with NaI in acetone into the oily S-γ-N-carbobenzoxy-L-alanyl-β-benzyl-α-iodohydrin (VIII); $[\alpha]_D^{24}$ —14.4° (c 4.4; benzene). The condensation of the iodide (VIII) with silver benzyl D-distearoyl-α-glycerylphosphate (IX) [13] yielded S-α-(distearoyl-D-α'-glyceryl-benzylphosphoryl)-β-benzyl-γ-(N-carbobenzoxy-L-alanyl)-glycerol (X), isolated in the form of a waxy substance with m.p. ~40° (from ether—methanol); $[\alpha]_D^{20}$ —2.9° (c 10; dioxane). The hydrogenolysis of the

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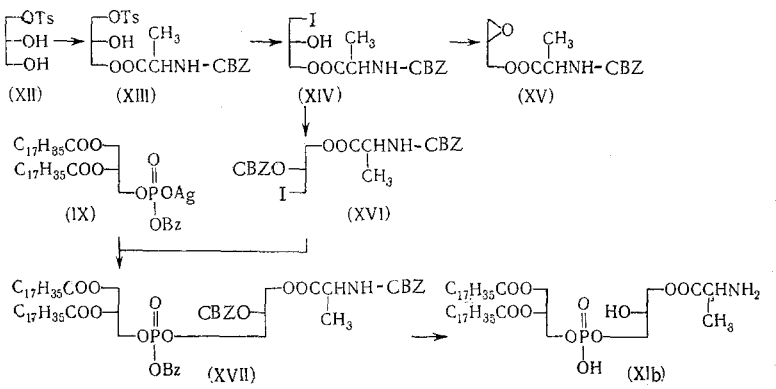
Scheme 2



phosphoric triester (X) over palladium in ethyl acetate in the presence of CH_3COOH gave S-α-(distearoyl-L-phosphatidyl)-γ-L-alanylglycerol (XIa) in the form of a colorless powder which, after crystallization from chloroform—acetone, sintered at ~55° and melted at 70-75°; $[\alpha]_D^{18}$ + 7.8° (c 1.5, chloroform).

The synthesis of the R-isomer of (XIa)-(XIb) — was effected by scheme 2. D-α-Tosylglycerol (XII) [14] was esterfied with N-carbobenzoxy-L-alanine in the presence of dicyclohexylcarbodiimide to form R-α-N-carbobenzoxy-L-alanyl-γ-tosylglycerol (XIII); m.p. 89-90° (from ether); $[\alpha]_D^{20}$ −18.7° (c 4.6; dioxane). When this was boiled with NaI in acetone, it formed R-γ-N-carbobenzoxy-L-alanyl-α-iodohydrin (XIV); m.p. 65-66° (from ether—petroleum ether); $[\alpha]_D^{21}$ −10.1° (c 3.8, dioxane). The γ-position of the carbobenzoxyalanyl grouping in (XIV) was confirmed by its conversion under mild conditions (under the action of dry Ag_2O in benzene at 20° for 5 h) into the S-2,3-epoxypropyl ester of N-carbobenzoxy-L-alanine (XV); $[\alpha]_D^{19}$ −2.3° (c 3.9, dioxane). The structure of (XV) was confirmed by its IR spectrum (a strong band characteristic for epoxides at 1260 cm^{-1}). By the action of $C_6H_5CH_2OCOCl$ and pyridine in ether on the iodohydrin (XIV) R-γ-N-carbobenzoxy-L-alanyl-β-carbobenzoxy-α-iodohydrin (XVI) was obtained in the form of an oil with $[\alpha]_D^{14}$ −13.0° (c 3.4; dioxane). When the derivative (XVI) was condensed with the silver salt (IX), the main reaction product was dibenzyl D-α,β-distearoylglyceryl phosphate (yield 70%), while the desired reaction product — R-α-(D-α',β'-distearoylglycerylbenzylphosphoryl)-β-carbobenzoxy-γ-N-carbobenzoxy-L-alanylglycerol (XVII) — was obtained with a yield of 8% in the form of a colorless wax-like substance with m.p. 38-39° (from ether—methanol); $[\alpha]_D^{20}$ −3.9° (c 9, dioxane).

On hydrogenolysis over palladium, the phosphoric triester (XVII) gave a substance which, after crystallization from a mixture of chloroform and acetone softened at ~80°, partially melted at 95°, and melted completely at 175°, $[\alpha]_D^{18}$ + 1.6° (c 0.7, chloroform). In spite of the fact that the product obtained gave a high carbon content on analysis,* we assume that it was the R-isomer of the lipoaminoacid (XIa)-(XIb), since the two substances had similar IR spectra. On thin-layer chromatography on silica gel in the diisobutyl ketone—acetic acid—water (40 : 25 : 5) system, (XIa) and (XIb) had similar R_f values (0.35 and 0.31), but in the chloroform—methanol—water (65 : 25 : 3) system, their R_f values were, respectively, 0.56 and 0.31.

At the present time we are carrying out a direct comparison of compounds (XIa) and (XIb) with the lipoaminoacid obtained from Clostridium welchii [1].

CONCLUSIONS

S-α-(Distearoyl-L-phosphatidyl)-γ-L-alanylglycerol and its R-isomer have been synthesized.

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