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SYNTHESIS OF OPTICAL ACTIVE LIPOAMINO ACIDS

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Previous studies have shown that the phospholipids of some bacteria contain alanine, glycine, lysine or ornithine in bound form (1-4). Since various investigators had suggested earlier that lipoamino acid complexes may be involved in protein synthesis by microorganisms as well as by higher plants and animals (5-8) these reports aroused considerable interest.

Analytical and spectral data indicated the bacterial lipoamino acids to be amino acid esters of phosphatidyl glycerol of the type (XI) (1-3), leaving unsettled the position of the amino acid residue and the configuration of the phosphatidyl glycerol moiety.

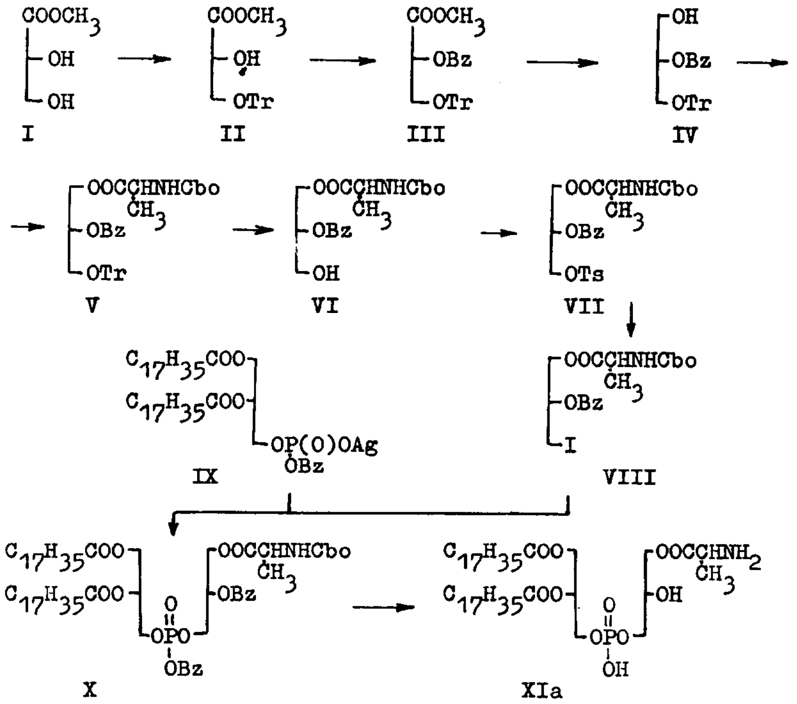
Recently two syntheses of compounds of type XI were reported. Since both syntheses afforded only partly or completely racemic compounds (9,10), the problem of the structure and configuration of the natural lipoamino acids remains still obscure.

Intending to investigate this question we have undertaken a synthesis of the two optical active epimers of α-(L-distearoylphosphatidyl)-γ-L-alanylglycerol (XIa) and (XIb) with different configuration of the L-alanyl glycerol moiety.

The synthesis of epimer XIa was accomplished as follows (see Scheme 1). Tritylation of methyl D-glycerate (I) (obtained from D-α,β-isopropylidene-glyceraldehyde by the method described for the corresponding racemate (11)) led to methyl D-β-O-tritylglycerate (II), m.p. 127-128° (from ether-hexane), $[\alpha]_D^{18}$ -5.3 (c 5.7; dioxane) (Found: C 76.36, H 6.22. Calcd. for $C_{23}H_{22}O_4$: C 76.22, H 6.12). Benzylation of II with benzyl bromide in the presence of silver oxide in benzene gave methyl R-α-O-benzyl-β-O-tritylglycerate (III) which without purification was reduced by $LiAlH_4$ to S-β-O-benzyl-γ-O-tritylglycerol (IV), m.p. 81-82° (from benzene-hexane), $[\alpha]_D^{23}$ -13.2° (c 4.2; dioxane) (Found: C 81.75, H 6.65. Calcd. for $C_{29}H_{28}O_3$: C 81.52, H 6.84). Esterification of IV with N-carbobenzoxy-L-alanine in the presence of dicyclohexylcarbodiimide led to R-α-(N-carbobenzoxy-L-alanyl)-β-O-benzyl-γ-O-tritylglycerol (V) as an oil with $[\alpha]_D^{22}$ -15.8° (c 5.5; dioxane) (Found: C 76.48, H 6.03, N 2.14. Calcd. for $C_{40}H_{39}O_6N$: C 75.29, H 6.24, N 2.22). Removal of the trityl group from V by hydrolysis on silicic acid (12,13) or by diluted hydrochloric acid in dioxane at room temperature afforded R-α-(N-carbobenzoxy-L-alanyl)-β-O-benzylglycerol (VI) (colourless syrup), $[\alpha]_D^{22}$ -12.5° (c 6.8; dioxane) (Found: N 3.43. Calcd. for $C_{21}H_{25}O_6N$: N 3.62).

Tosylation of VI with tosyl chloride in the presence of pyridine yielded S-α-(N-carbobenzoxy-L-alanyl)-β-O-benzyl-

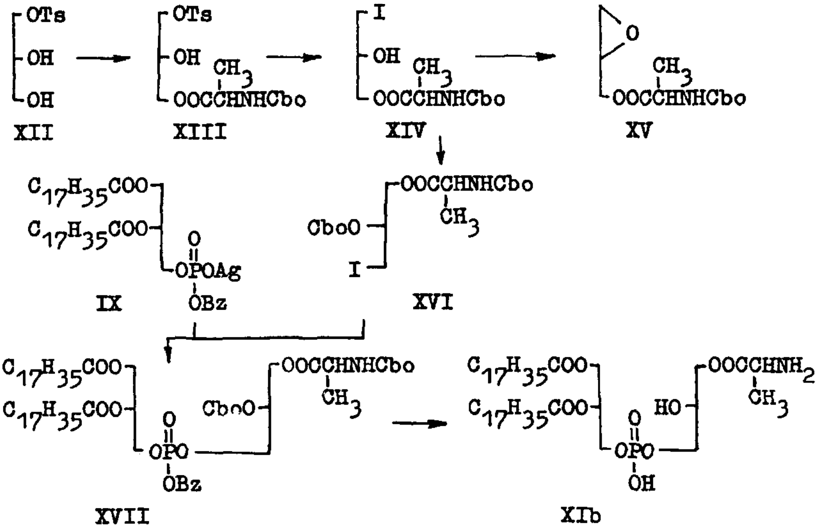
Scheme 1



-γ-tosylglycerol (VII) which without purification was converted by sodium iodide in boiling acetone into the corresponding iodide (VIII) (oil), $[\alpha]_D^{24}$ -14.4° (c 4.4; benzene) (Found: C 51.24, H 5.06, I 25.18. Calcd. for $C_{21}H_{24}O_5NI$: C 50.70, H 4.86, I 25.52). Condensation of VIII with silver benzyl D-α,β-distearoylglycerol phosphate (IX) (14) yielded S-α-[(D-α′,β′-distearoylglyceryl)-benzylphosphoryl]-β-O-benzyl-γ-(N-carbobenzoxy-L-alanyl)-glycerol (X) as a wax-like substance with m.p. about 40° (from ether-MeOH), $[\alpha]_D^{20}$ -2.9°

(c 10; dioxane) (Found: C 69.04, H 9.08, N 1.43, P 2.34. Calcd. for $C_{67}H_{106}O_{13}NP$: C 69.10, H 9.18, N 1.20, P 2.26). Hydrogenolysis of the phosphoric triester X with palladium as a catalyst in ethyl acetate in the presence of glacial acetic acid yielded S-α-(distearoyl-L-phosphatidyl)-γ-L-alanylglycerol (XIa) as a colourless powder sintering after crystallisation from chloroform-acetone at 55° and melting at 70-75°, $[\alpha]_D^{18}$+7.8° (c 1.5; chloroform) (Found: C 63.90, H 10.30, N 1.58, P 3.79. Calcd. for $C_{45}H_{88}O_{11}NP$: C 63.57, H 10.43, N 1.65, P 3.65).

The synthesis of the R-isomer of XIa (XIb) was carried out by the reaction sequence depicted on Scheme 2. D-α-Tosylglycerol (XII) was esterified with N-carbobenzoxy-L-alanine in the presence of dicyclohexylcarbodiimide to give R-α-(N-carbobenzoxy-L-alanyl)-γ-tosylglycerol (XIII), m.p. 89-90° (from ether), $[\alpha]_D^{20}$ -18.7° (c 4.6; dioxane) (Found: C 56.01, H 5.52, N 3.14, S 6.91. Calcd. for $C_{21}H_{25}O_6NS$: C 55.87, H 5.58, N 3.10, S 7.10). After boiling with sodium iodide in acetone XIII yielded R-α-(N-carbobenzoxy-L-alanyl)-glycerol-γ-iodohydrin (XIV), m.p. 65-66° (from ether-hexane), $[\alpha]_D^{21}$ -10.1° (c 3.8; dioxane) (Found: C 41.84, H 4.75, I 31.73. Calcd. for $C_{14}H_{18}O_5NI$: C 41.29, H 4.46, I 31.17). The α-position of the N-carbobenzoxy-L-alanyl residue in XIV was confirmed by the conversion of the iodohydrin under mild conditions (treatment with dry silver oxide in benzene during 5 hours at room temperature) into S-2,3-oxido-(N-carbobenzoxy-L-alanyl)-propanol (XV) (oil), $[\alpha]_D^{19}$ -2.3° (c 3.9; dioxane) (Found: C 60.13, H 6.25, Scheme 2



N 4.84. Calcd. for $C_{14}H_{17}O_5N$: C 60.20, H 6.14, N 5.02). The IR-spectrum of XV revealed a strong epoxide band at 1260 cm^{-1}. The iodohydrin XIV was treated with carbobenzoxy chloride and pyridine in ether to afford R-α-(N-carbobenzoxy-L-alanyl)-β-O-carbobenzoxyglycerol-γ-iodohydrin (XVI) as a nearly colourless oil with $[\alpha]_D^{14}$ -13.0° (c 3.4; dioxane) (Found: C 48.83, H 4.64, N 2.59, I 23.16. Calcd. for $C_{22}H_{24}O_7NI$: C 48.81, H 4.47, N 2.59, I 23.44). The main product of the condensation of iodohydrin XVI with the silver salt IX was dibenzyl D-α,β-distearoylglycerol phosphate (yield 70%), while the yield of the necessary substance R-α-[(D-α′,β′-distearoylglyceryl)-benzylphosphoryl]-β-O-carbobenzoxy-γ-(N-carbobenzoxy-L-alanyl)-glycerol (XVII) accounted to 8% only, m.p.

38-39° (from ether-MeOH), $[\alpha]_D^{20}$ -3.9° (c 9; dioxane) (Found: C 67.41, H 9.13, N 1.18, P 2.43. Calcd. for $C_{68}H_{106}O_{15}NP$: C 67.58, H 8.84, N 1.15, P 2.57).

Hydrogenolysis of the triphosphate XVII, accomplished as described for X, yielded a substance, sintering after crystallisation from chloroform-acetone at 80°, melting partially at 95° and melting completely at 175°, $[\alpha]_D^{18}$+1.6° (c 0.7; chloroform) (Found: N 1.39, P 3.82. Calcd. for $C_{45}H_{88}O_{11}NP$: N 1.65, P 3.65). Although the carbon value for the obtained product was too low, we believe that it must be the R-isomer of XIa (XIb) as the IR-spectra of both substances are almost identical. On silicic acid TLC XIa and XIb were found to have very close R_f values in the system diisobutyl ketone-CH_3COOH-H_2O 40:25:5 (0.35 and 0.31), but the R_f values in the system $CHCl_3$-MeOH-H_2O 65:25:3 differ considerably (0.56 and 0.31 respectively).

The comparision of the compounds XIa and XIb with the lipoamino acid obtained from Clostridium Welchii (1) is in progress.

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