Chem. Phys. Lipids 2 (1968) 1–10 © North-Holland Publ. Co., Amsterdam

ON THE STRUCTURE OF LIPOAMINO ACIDS

JUL. G. MOLOTKOVSKY and L. D. BERGELSON

Institute for Chemistry of Natural Products,
USSR Academy of Sciences, Moscow

Received 3 October 1967

Syntheses of 1-[(1′,2′-distearoyl-glyceryl)-phosphoryl]-3-L-lysyl-glycerol (XII) and 1-[(1′,2′-distearoyl-glyceryl)-phosphoryl]-2-L-lysyl-glycerol (XXII), as well as of 1-L-lysyl-3-[(1′,2′-distearoyl-glyceryl)-phosphoryl]-glycerol (XVI) are described, the first two substances having the same configuration at all asymmetric atoms as the natural lysyl ester of phosphatidyl glycerol.

It was shown that the α- and β-lysyl-γ-phosphatidyl-glycerols (XII) and (XXII) differ by their melting points and chromatographic properties and that they do not undergo interconversion on silica gel chromatography in acid or neutral systems. Since synthetic 1-[(1′-oleoyl-2′-palmitoyl-glyceryl)-phosphoryl]-3-L-lysyl-glycerol was shown to be chromatographically indistinguishable from the natural lipoamino acid (P. P. M. Bonsen, G. H. de Haas and L. L. M. van Deenen, Biochemistry *6* (1967) 1114), the native compound must be the α-isomer.

The synthesis of two stereoisomeric α-alanyl-γ-phosphatidyl-glycerols, namely 1-L-phosphatidyl-3-L-alanyl-glycerol (I) and 1-L-alanyl-3-L-phosphatidyl-glycerol (II)* (fig. 1) was reported from this laboratory earlier[1,2]). Comparision of these compounds with the lipoamino acid from *Clostridium welchii*[4]) showed the synthetic S-isomer (I) to be chromatographically very close to the natural compound, while the synthetic R-isomer (II) was quite different.

This result appeared to confirm the work of van Deenen et al.[5,6]) on the

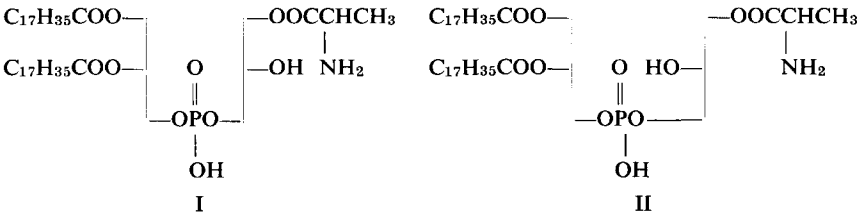


Fig. 1

* For optically active glycerol derivatives the Hirschmann nomenclature[3]) is used.

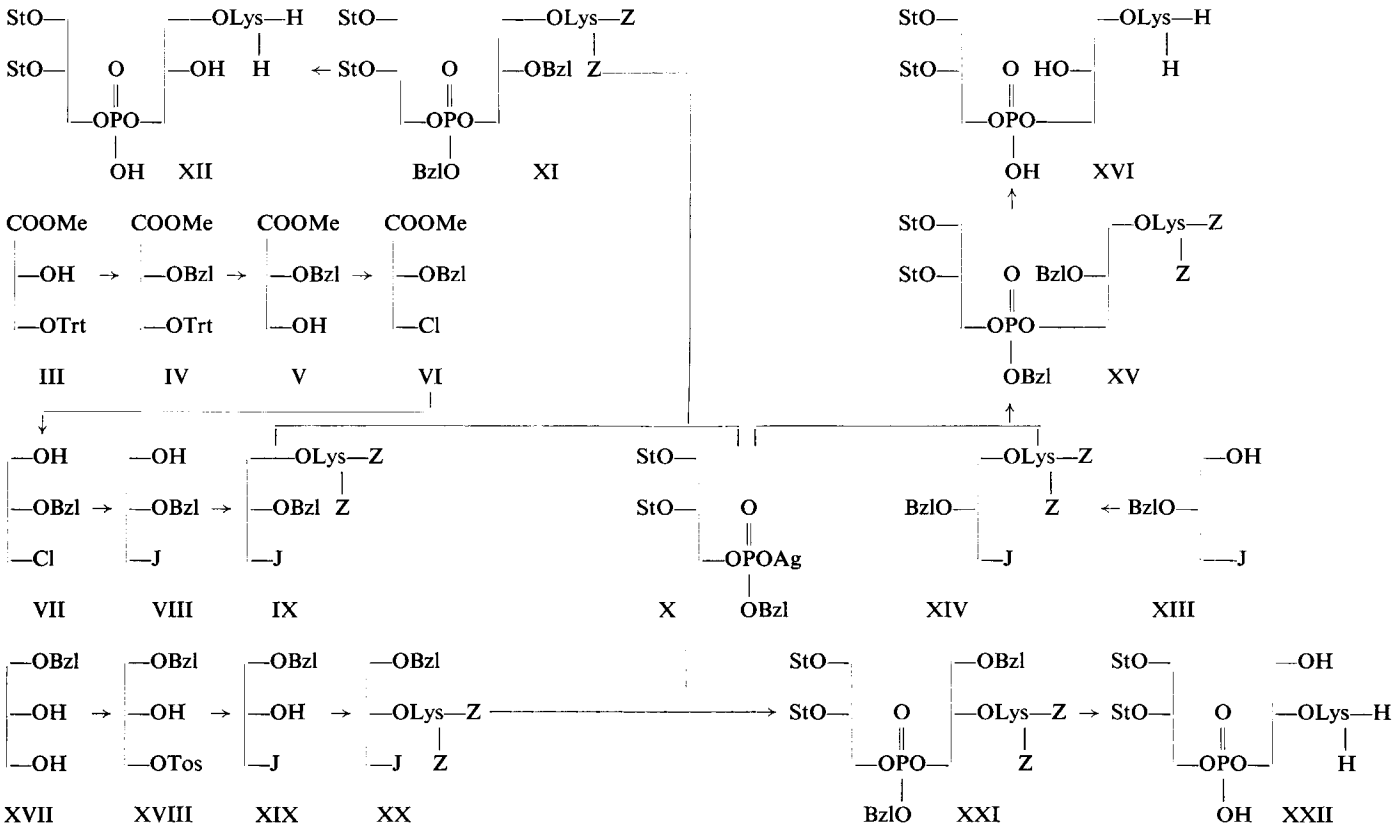
enzymic splitting of synthetic 1-[(1′-oleoyl-2′-palmitoyl-glyceryl)-phosphoryl]-3-L-lysyl-glycerol and the lysyl ester of phosphatidyl glycerol from *Staphylococcus aureus*, on the ground of which these authors proposed the S-configuration for the amino acid bound glycerol residue in the bacterial lipoamino acids.

However despite of the identity of the natural and synthetic preparations investigated in van Deenen's laboratory, the location of the amino acid in the glycerol chain remained still unresolved, since the migration of the amino acid residue from the β- into the α-position during the isolation and chromatographic purification procedure could not be excluded[7]). Such a migration appeared to be possible on the ground of the data of Shabarova et al.[8]) and Khorana et al.[9]), which showed the extreme lability of amino acid residues in nucleosides in acidic and neutral media.

One of the possible approaches to solve this problem could be a study of the stability and of the optical and chromatographic properties of the synthetic 2- and 3-amino acid esters of 1-L-phosphatidyl glycerol and their comparision with the natural lipoamino acids. To achieve this we carried out the syntheses of 1-L-phosphatidyl-3-L-lysyl-glycerol (XII) and its β-substituted isomer (XXII), as well as the R-isomer of XII – 1-L-lysyl-3-L-phosphatidyl-glycerol (XVI). All three isomers were synthesized by similar routes, based on the condensation of silver benzyl (1,2-distearoyl-glyceryl)-phosphate (X) with the corresponding N,N′-dicarbobenzoxy-L-lysyl esters of the isomeric 0-benzyl-glycerol iodohydrins (IX, XIV and XX) (see fig. 2).

An attempt to obtain the iodide (IX) necessary for the synthesis of the S-lipoamino acid (XII), by the scheme shown on fig. 3, earlier applied in the synthesis of the alanine analogue (I)[1]), was unsuccessful, because it turned out to be impossible to hydrolyze the fully protected compound (XXIV) to the hydroxyester (XXV) under mild conditions (on silica gel or by dilute HCl in aqueous dioxane at room temperature). On the other hand detritylation of XXIV by dilute HCl in aqueous dioxane at 50–60° caused migration of the lysyl grouping from position 3 into position 1 accompanied by partial racemization as was shown by retritylation of XXV to XXIV.

The iodide (IX) was therefore synthesized as shown in fig. 2, starting from methyl 1-0-trityl-3-glycerate (III)[1]). Benzylation of the latter gave the 0-benzyl derivative (IV) forming methyl 2-0-benzyl-3-glycerate (V) on acid detritylation. Treatment of V with thionyl chloride in pyridine led to the chloride (VI) transformed by $LiAlH_4$ reduction to the chlorohydrin (VII). The latter without purification was converted into the iodohydrin (VIII) by treatment with sodium iodide in acetone. Esterification of the iodohydrin (VIII) to the lysyl derivative (IX) was carried out by treatment with N, N′-dicarbobenzoxy-L-lysine[10]) and dicyclohexylcarbodiimide.



St = stearoyl; for other abbreviations see IUPAC-IUB Tentative Rules[15])

Fig. 2

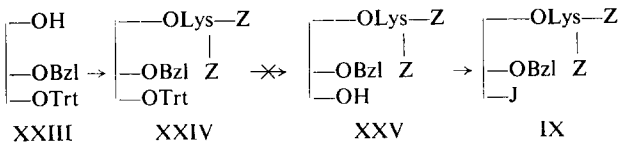


Fig. 3

To obtain the lipoamino acid (XXII) with the secondary hydroxy group esterified by lysine, it was necessary to synthesize 2-(N,N′-dicarbobenzoxy-L-lysyl)-3-0-benzyl-glycerol-1-iodohydrin (XX). The starting compound for this synthesis was 3-0-benzyl-glycerol (XVII)[11]) transformed through the monotosylate (XVIII) to 3-0-benzyl-glycerol-1-iodohydrin (XIX). Esterification of the latter was carried out by the mixed acid anhydride method with N,N′-dicarbobenzoxy-L-lysine and benzenesulfonyl chloride.

The synthesis of the R-lipoamino acid (XVI) was achieved starting from the antipode of VIII – the iodide (XIII)[2]), the latter giving the N,N′-dicarbobenzoxy-L-lysyl derivative (XIV) by carbodiimide esterification.

The fully protected lipoamino acids (XI, XV and XXI), obtained by condensation of the corresponding iodides (IX, XIV and XX) with the silver salt (X) were hydrogenated over $Pd/BaSO_4$[12]) in dioxane whereby the lipoamino acids (XII, XVI and XXII) were obtained in 30–40% yields as colourless powders. Their melting points and optical rotation values as well as the melting points of the dihydrochlorides are presented in table 1.

TABLE 1

Physical data of lipoamino acids and their dichlorohydrates.

Lipoamino acid	M.p. (with decomposition)	$[\alpha]_D$ in chloroform	M.p. of dichlorohydrate (decomp.)
XII	104–106° (sinters ca. 70°)	+ 5.5° (c 1.9)	203–205° (sinters 100–110°)
XVI	194–198° (sinters 100–110°)	+ 14.8° (c 1.1)	201–203° (sinters 100–110°)
XXII	109–112° (sinters ca. 70°)	+ 3.0° (c 1.3)	198–200° (sinters 100–110°)

As can be seen from table 1 the α- and β-lysyl-γ-phosphatidyl-glycerols (XII) and (XXII) obviously differ in the melting points of bases and dihydrochlorides. On silica gel TLC (see table 2) in diisobutylketone-acetic acid-water (40:25:5 v/v) the α-lipoamino acid (XII) revealed a lower R_f value than the β-isomer. Mixtures of the α- and β-isomers were resolved into the single components under the above conditions.

The results obtained show that the β-lipoamino acid (XXII) is sufficiently stable to be isolated by silica gel chromatography in acid or neutral systems

TABLE 2

R_f values of lipoamino acids on TLC. Technique: ascending; spray: ninhydrin.

Substance	Developing system Chloroform–methanol–water (65:25:4 v/v)	Chloroform–methanol–14% NH_3 (17:7:1 v/v)	Di-isobutyl ketone–acetic acid–water (40:25:5 v/v)
XII	0.37	0.27*	0.28
XVI	0.38	0.27*	0.33
XXII	0.38	0.26*	0.33
0-lysyl ester of phosphatidyl glycerol from *St. aureus*[5]**	0.36	—	0.26

* with tailing
** added in proof

and that it is not transformed into the α-isomer under the above conditions. Therefore the earlier expressed apprehension[7]) of a possible rearrangement of lipoamino acids during the isolation and purification procedures seems to be unjustified. Since the synthetic 1-L-phosphatidyl-3-L-lysyl-glycerol was shown[6]) to be chromatographically indistinguishable from the lipoamino acid from *Staphylococcus aureus*, it now can be regarded as finally proved that the latter is the α-isomer. Probably this also holds for the lipoamino acids isolated from other sources.

EXPERIMENTAL PART

General methods

Melting points were determined on a Kofler block and corrected. For the lipoamino acids (XII, XVI and XXII) and their dichlorohydrates the temperatures of menisk formation in sealed capillaries under argon were regarded as melting points (*cf.*[13])). Optical rotations were measured on a Hilger polarimeter at 17–22° in dioxane solutions (except some specified cases); IR spectra were recorded on a UR-10 spectrophotometer (Karl Zeiss, Jena). TLC was carried out on KSK silica gel (<150 mesh) with 5% $CaSO_4$ as binder; charring with conc. H_2SO_4 was used for detection. Column chromatography was carried out on KSK silica gel (150–250 mesh), gradient elution being used in all cases. Petroleum ether b.p. 40–60° was used.

Methyl 1-0-trityl-2-0-benzyl-3-glycerate (IV)

A solution of 6.3 g of methyl 1-0-trityl-3-glycerate (III)[1]) and 2.4 ml of benzyl bromide in 50 ml of dry benzene was stirred for 6 hr at room temperature in the dark with 9.5 g of freshly prepared dry silver oxide. The mixture was filtered, the precipitate washed with ethyl acetate and the combined filtrates were evaporated. The oily residue was chromatographed in pet.

ether–ether on a column loaded with 250 g of silica gel, fraction control being conducted by TLC in pet. ether–ether (4:1 v/v). 15–20% ether in pet. ether eluted IV as a colourless syrup in 64% yield, $[\alpha]_D + 30.4°$ (c 8.5). IR spectrum (film): 1758 (s), 1601 (m), 1205 (s) cm^{-1}.

Found C 79.48 H 6.47
Calcd. for $C_{30}H_{28}O_4$ C 79.62 H 6.24.

Methyl 2-0-benzyl-3-glycerate (V)

A solution of 3.8 g of the ester (IV) in 200 ml of methanol was treated with 0.4 ml of conc. HCl at room temperature for 10 hr. The mixture was diluted with ether (300 ml), washed successively with $NaHCO_3$aq., water and brine, dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed on 200 g of silica gel in benzene–ethyl acetate, the fractions being checked by TLC in benzene–ethyl acetate (4:1 v/v). V was eluted in 75% yield with 15–20% ethyl acetate in benzene as a thick oil with b.p. 150° (bath)/0.2 mm, $[\alpha]_D + 75.3°$ (c 5.8). IR spectrum (film): 3500 (s; broad), 1750 (s), 1208 (s), 1127 (s) cm^{-1}.

Found C 62.91 H 6.70
Calcd. for $C_{11}H_{14}O_4$ C 62.84 H 6.71.

Methyl S-α-benzyloxy-β-chloropropionate (VI)

A solution of 0.43 ml of thionyl chloride in 2 ml of dry chloroform was added to a mixture of 1.2 g of the hydroxyester (V), 0.55 ml of pyridine and 5 ml of dry chloroform and the solution was set aside for 2 days at room temperature. The mixture was diluted with ether (50 ml), washed in succession with $NaHCO_3$aq., water, 1 N HCl, water and brine, dried over $MgSO_4$ and filtered. Evaporation gave 1.15 g (88%) of VI as a pale yellow oil pure on TLC. B.p. 140°(bath)/0.2 mm, $[\alpha]_D + 66.3°$ (c 3.9). IR spectrum (film): 1755 (s), 1215 (s), 1125 (s) cm^{-1}.

Found C 58.05 H 5.80 Cl 15.40
Calcd. for $C_{11}H_{13}ClO_3$ C 57.77 H 5.73 Cl 15.51.

2-0-Benzyl-glycerol-1-iodohydrin (VIII)

A solution of 0.8 g of the chloroester (VI) in 10 ml of ether was added during 10 min to a suspension of 0.12 g of $LiAlH_4$ in 30 ml of ether. The mixture was stirred for 2 hr, 1 ml of ethyl acetate was added carefully, and after 5 min the mixture was acidified by 20 ml of 5% H_2SO_4. The upper layer was washed successively by water, $NaHCO_3$aq., water and brine, dried over $MgSO_4$, filtered and evaporated. The crude 2-0-benzyl-glycerol-1-chlorohydrin (VII) obtained as a thick oil (0.8 g) was dissolved in 25 ml of dry acetone and heated with 1 g of dry sodium iodide in a sealed tube at 90°

for 17 hr in the dark. The resulting mixture was diluted with 100 ml of benzene, washed with 1% $Na_2S_2O_3$, water and brine, dried over $MgSO_4$, filtrated and evaporated. Chromatography of the residue on 120 g of silica gel in benzene with ether (control on TLG in benzene–ether 2:1) gave 0.43 g (42%) of the iodide (VIII) as a pale yellow oil, $[\alpha]_D - 5.3°$ (c 7.2). [The R-isomer[2]) has $[\alpha]_D + 4.9°$ (c 7)]. IR spectrum (film): 3420 (s; broad), 1065 (s) cm^{-1}.

Found C 40.91 H 4.64 I 42.79
Calcd. for $C_{10}H_{13}IO_2$ C 41.20 H 4.48 I 43.39.

2-0-Benzyl-3-(N,N'-dicarbobenzoxy-L-lysyl)-glycerol-1-iodohydrin (IX)

A solution of 300 mg of dicyclohexylcarbodiimide in 1 ml of pyridine was added dropwise to an ice-cooled stirred solution of 255 mg of the iodide (VIII) and 450 mg of N,N'-dicarbobenzoxy-L-lysine[10]) in 2 ml of pyridine, the mixture was stirred for 5 hr at 0° and set aside for 2 days at room temperature. Ether (5 ml) was added to the mixture, the precipitate of dicyclohexylurea was filtered off and washed with ether. After evaporating of the filtrate the residue was chromatographed on a column, packed with 100 g of alumina (III-IV grade, neutral) in benzene. Elution with 10–15% ethyl acetate in benzene gave 530 mg (88%) of the iodide (IX) as a syrup, $[\alpha]_D -$ 6.8° (c 6.2). IR spectrum (film): 3350 (s), 1714 (s; broad), 1530 (s), 1253 (s), 1030 (s) cm^{-1}.

Found C 56.08 H 5.59 N 4.32
Calcd. for $C_{32}H_{37}IN_2O_7$ C 55.82 H 5.42 N 4.07.

1-(N,N'-Dicarbobenzoxy-L-lysyl)-2-0-benzyl-glycerol-3-iodohydrin (XIV)

120 mg of 2-0-benzyl-glycerol-3-iodohydrin (XIII)[2]) was reacted with N,N'-dicarbobenzoxy-L-lysine, as described for IX; 290 mg of a gum, crystallizing on standing were obtained. Crystallization from ether–pet. ether gave 200 mg (71%) of XIV as needles, m.p. 90–91.5°, $[\alpha]_D - 5.1°$ (c 5.3). IR spectrum (KBr): 3360 (s), 1774 (s), 1692 (s), 1538 (s), 1032 (s) cm^{-1}

Found C 55.66 H 5.19 N 4.11
Calcd. for $C_{32}H_{37}IN_2O_7$ C 55.82 H 5.42 N 4.07.

3-0-Benzyl-glycerol-1-iodohydrin (XIX)

0.95 g of tosyl chloride was added to an ice-cooled solution of 0.55 g of 3-0-benzyl-glycerol (XVII)[11]), the mixture was stored in the refrigerator overnight, diluted with ether, washed in succession with $NaHCO_3$aq., water, 1 N HCl, water and brine and dried over $MgSO_4$. After evaporation the residue was dissolved in 15 ml of dry acetone and treated with 1 g of dry sodium iodide in a sealed tube at 80° for 6 hr in the dark. After the routine

treatment the reaction product was chromatographed on a column packed with 40 g of silica gel in benzene, checking of fractions being conducted by TLC in benzene–ethyl acetate (5:1 v/v). Elution with 15–20% ethyl acetate in benzene gave 0.65 g (74%) of XIX as a pale yellow oil, $[\alpha]_D + 2.2°$ (c 8.7). IR spectrum (film): 3340 (s; broad), 1106 (s) cm^{-1}.

Found C 41.63 H 4.72 I 42.80

Calcd. for $C_{10}H_{13}IO_2$ C 41.20 H 4.48 I 43.39.

2-(N,N'-Dicarbobenzoxy-L-lysyl)-3-0-benzyl-glycerol-1-iodohydrin (XX)

0.123 ml of benzenesulphonyl chloride was added with stirring at −5° to a solution of 0.4 g of N,N'-dicarbobenzoxy-L-lysine in 2 ml of pyridine. After stirring for 10 min a solution of 140 mg of the iodohydrin (XIX) in 0.5 ml of pyridine was added and the solution was left overnight at room temperature. After dilution with benzene (50 ml) the mixture was washed successively with 1% $Na_2S_2O_3$, 1 N HCl, water and brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on a column, filled with 40 g of silica gel in benzene, the eluates were checked by TLC in benzene–ether (3:1 v/v). Elution with 15–20% ether in benzene gave 300 mg of a gum, crystallizing on standing. Crystallization from ether–pet. ether gave 258 mg (78%) of XX as needles, m.p. 80–81°, $[\alpha]_D$ 0° (c 5.2). IR spectrum (KBr): 3340 (s), 1740 (s), 1690 (s), 1535 (s) cm^{-1}.

Found C 55.94 H 5.48 I 19.04 N 4.34

Calcd. for $C_{32}H_{37}IN_2O_7$ C 55.82 H 5.42 I 18.43 N 4.07.

1-[(1',2'-Distearoyl-glyceryl)-benzyl-phosphoryl]-2-0-benzyl-3-(N,N'-dicarbobenzoxy-L-lysyl)-glycerol (XI)

A mixture of 130 mg of the iodide (IX), 180 mg silver (1,2-distearoyl-glyceryl)-benzyl-phosphate (X)[14]) and 5 ml of dry benzene was sealed in a tube under argon and kept for 4 hr at 90° in the dark. The mixture was centrifugated, the silver iodide precipitate washed with 3 ml of benzene and again centrifugated. The united supernatants were evaporated and the residue was chromatographed on 20 g of silica gel (fraction control by TLC in benzene–ether, 1:1 v/v). Elution with 20–25% ethyl acetate in benzene gave 115 mg (45%) of the triphosphate (XI) as a wax with m.p. 36–37°, $[\alpha]_D - 0.7°$ (c 5.8).

Found C 68.78 H 9.01 N 2.08 P 1.98

Calcd. for $C_{78}H_{119}N_2O_{15}P$ C 69.09 H 8.85 N 2.08 P 2.28.

1-(N,N'-Dicarbobenzoxy-L-lysyl)-2-0-benzyl-3-[(1',2'-distearoyl-glyceryl)-benzyl-phosphoryl]-glycerol (XV)

This substance was obtained as described for XI, starting from 120 mg of

the iodide (XIV) and 165 mg of the silver salt (X), the yield of the wax-like triphosphate was 43%, m.p. 35–36°, $[\alpha]_D + 1.4°$ (c 7.1).

Found C 68.82 H 8.82 N 2.38 P 1.95

Calcd. for $C_{78}H_{119}N_2O_{15}P$ C 69.09 H 8.85 N 2.08 P 2.28.

1-[(1',2'-Distearoyl-glyceryl)-benzyl-phosphoryl]-2-(N,N'-dicarbobenzoxy-L-lysyl)-3-0-benzyl-glycerol (XXI)

130 mg of the iodide (XX) and 180 mg of the silver salt (X) gave in the same manner XXI in 69% yield as a wax-like substance with m.p. 34–35°, $[\alpha]_D + 3.8°$ (c 5.8).

Found C 68.72 H 9.12 N 2.32 P 2.25

Calcd. for $C_{78}H_{119}N_2O_{15}P$ C 69.09 H 8.85 N 2.08 P 2.28.

1-[(1',2'-Distearoyl-glyceryl)-phosphoryl]-3-L-lysyl-glycerol (XII)

100 mg of 5% $PdO/BaSO_4$ [12]), suspended in a mixture of 5 ml of dioxane and 0.1 ml glacial acetic acid was pre-reduced, 105 mg of the triphosphate (XI) was added and the mixture was hydrogenated for 10 hr. Judging by TLC (system chloroform–methanol–water, 65:25:4 v/v; spray reagent ninhydrin), the reaction mixture contained two main components with $R_f \sim 0.7$ and ~ 0.4. The mixture was chromatographed on a column loaded with 10 g silica gel, elution with 15–20% methanol in chloroform gave 22 mg of a

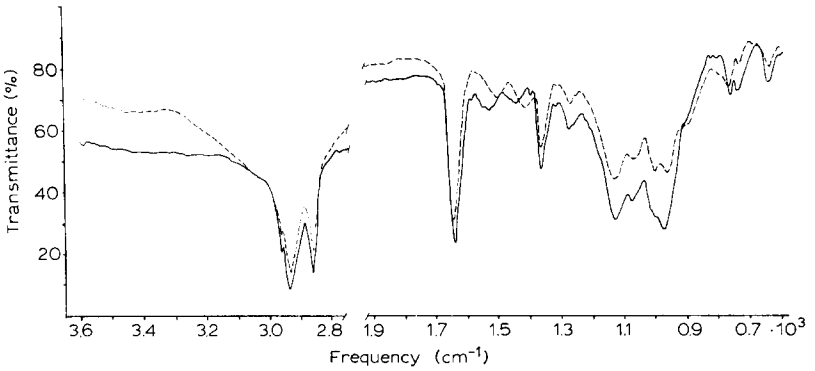


Fig. 4. IR spectra (KBr discs) of 1-[(1',2'-distearoyl-glyceryl)-phosphoryl]-3-L-lysyl-glycerol (XII) (———) and 1-[(1',2'-distearoyl-glyceryl)-phosphoryl]-2-L-lysyl-glycerol (XXII) (– – – –).

substance with $R_f \sim 0.7$, that did not change after additional hydrogenolysis and was not investigated further. Mixtures of chloroform–methanol (1:2–1:3 v/v) eluted the lipoamino acid as a colourless powder, crystallized from chloroform–acetone; yield 30 mg (43%). For data of XII see table 1. IR spectrum, see fig. 4.

Found C 63.14 H 10.43 N 3.18 P 3.35

Calcd. for $C_{48}H_{95}N_2O_{11}P$ C 63.53 H 10.56 N 3.09 P 3.43.

An ice-cooled solution of 2 mg of the lipoamino acid (XII) in 0.2 ml of mixture of chloroform–methanol (2:1 v/v) was treated with one drop of methanol–conc. HCl (9:1 v/v). Then 1 ml of acetone was added and the mixture was chilled at $-5°$ for 1 hr. The dichlorohydrate was separated by centrifugation, washed with acetone and dried over KOH pellets at 0.1 mm; for m.p. see table 1.

1-L-Lysyl-3-[(1',2'-distearoyl-glyceryl)-phosphoryl]-glycerol (XVI)

This lipoamino acid was obtained by the method described above from 98 mg of the triphosphate (XV) in a yield of 37%. For data of XVI and its dichlorohydrate see table 1. The IR spectra of XVI and XII are nearly identical.

Found C 63.25 H 10.27 N 3.18 P 3.55

Calcd. for $C_{48}H_{95}N_2O_{11}P$ C 63.53 H 10.56 N 3.09 P 3.43.

1-[(1',2'-Distearoyl-glyceryl)-phosphoryl]-2-L-lysyl-glycerol (XXII)

Hydrogenolysis of 102 mg of the triphosphate (XXI) accomplished in the same manner yielded 25 mg (37%) of the lipoamino acid (XXII); data listed in table 1. For IR spectrum see fig. 4.

Found C 62.93 H 10.53 N 2.90 P 3.64

Calcd. for $C_{48}H_{95}N_2O_{11}P$ C 63.53 H 10.43 N 3.09 P 3.43.

References

1) Jul. G. Molotkovsky and L. D. Bergelson, Izvest. Acad. Nauk SSSR, Otdel. Khim. Nauk (1967) 2321
2) Jul. G. Molotkovsky and L. D. Bergelson, Izvest. Acad. Nauk SSSR, Otdel. Khim. Nauk (1967) 2498
3) H. Hirschmann, J. Biol. Chem. **235** (1960) 2762
4) M. G. Macfarlane, Nature **196** (1963) 136
5) U. M. T. Houtsmuller, L. L. M. van Deenen, Biochim. Biophys. Acta **106** (1965) 564
6) P. P. M. Bonsen, G. H. de Haas, L. L. M. van Deenen, Biochemistry **6** (1967) 1114
7) P. P. M. Bonsen, Thesis, Utrecht (1967)
8) Z. A. Shabarova, N. A. Hughes, J. Baddiley, Biochem. J. **83** (1962) 216
9) D. H. Rammler, H. G. Khorana, J. Am. Chem. Soc. **85** (1963) 1997
10) R. A. Boissonas, S. Guttmann, R. L. Huguenin, P. A. Jaquenoud, E. Sandrin, Helv. Chim. Acta **41** (1958) 1867
11) J. C. Sowden, H. O. L. Fischer, J. Am. Chem. Soc. **63** (1941) 3244
12) R. Kuhn, H. J. Haas, Angew. Chemie **67** (1955) 785
13) E. Baer, B. C. Pal, Can. J. Biochem. **45** (1967) 309
14) E. Baer and S. K. Pavanaran, J. Biol. Chem. **236** (1961) 2410
15) Biochem. J. **102** (1967) 23