bp 60–61° (0.2 mm), $n^{25}D$ 1.5603. The H^1 nmr showed multiplets centered at τ 6.15 (4 H) and at 8.35 (6 H).

Anal. Calcd for $C_9H_{10}N_2OS$: C, 41.07; H, 6.91; N, 19.16; S, 21.93. Found: C, 41.02; H, 6.78; N, 18.97; S, 21.67.

p-Dimethylaminothionylaniline. Thionylaniline, 13.9 g (0.1 mole), was added to a solution of 13.0 g (0.1 mole) of practical N,N-dimethylphenylenediamine in 100 ml of ether. The black mixture was heated at reflux for 10 min, and then cooled to −78°. The solid that separated was collected on a cold filter and washed with pentane. Recrystallization from hexane gave 4.7 g of *p*-dimethylaminothionylaniline as red-orange plates, mp 70–73°. The H^1 nmr spectrum in CCl_4 showed a singlet at τ 7.07 ($N(CH_3)_2$) and an A_2B_2 pattern with components centered at about 2.27 and 3.56. The visible spectrum showed $\lambda_{max}^{cyclohexane}$ 415 mμ (ε 29,000).

Anal. Calcd for $C_8H_{10}N_2OS$: C, 52.73; H, 5.53; N, 15.37; S, 17.59. Found: C, 53.01; H, 5.45; N, 15.26; S, 17.34.

N-Thionitrosodimethylamine. A. By Reduction of Dimethylthionylhydrazine. A stirred solution of 26.5 g (0.25 mole) of dimethylthionylhydrazine in 100 ml of ether was cooled to −75°, and 63 ml of a 1 *M* solution (0.063 mole) of lithium aluminum hydride in ether was added dropwise over a period of 1 hr. The reaction mixture was allowed to warm to room temperature, stirred for 1 hr, and then filtered under nitrogen. The purple filtrate was concentrated by distillation at room temperature and reduced pressure (0.01 mm) until no further distillate came over. The deep purple residue was dissolved in 25 ml of ether, and the solution was filtered. The filtrate was cooled to −78°, and the crystals that separated were collected on a cold filter, washed with cold ether, and dried under a stream of nitrogen in a pressure filter. There was obtained 4.9 g of thionitrosodimethylamine as deep purple crystals, mp 20–21°. The infrared spectrum contained strong bands at 6.78, 7.45, and 9.05 μ with weaker bands at 3.40, 6.91, 7.95, 9.40, and 10.92 μ. The H^1 nmr spectrum of a 10% solution in carbon tetrachloride showed two singlets at τ 5.90 and 6.40.

Anal. Calcd for $C_2H_6N_2S$: C, 29.14; H, 6.94; N, 31.02; S, 35.12; mol wt, 90.15. Found: C, 28.65; H, 6.67; N, 31.08; S, 35.57; mol wt (freezing point in benzene), 90.

B. By Reaction of Sulfur with Dimethylhydrazine. A mixture of 400 ml of ether, 64 g (2 g-atoms) of powdered rhombic sulfur, and 60 g (1 mole) of 1,1-dimethylhydrazine was stirred at room temperature (*ca.* 25°) for 6 days. The reaction mixture slowly became deep purple during this time. The reaction mixture was filtered, and 35 g of undissolved sulfur was recovered. The purple filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized from ether at low temperature (−78°), and then dried under vacuum at 0° to give 13.65 g (15% conversion) of N-thionitrosodimethylamine, mp 20–21°. The product was soluble in water to give an orange solution, which could be extracted with ether to give a purple ethereal solution.

N-Thionitrosopiperidine. A stirred solution of 9.7 g (0.067 mole) of N-thionylaminopiperidine in 25 ml of ether was cooled to −50°, and 18 ml of 1 *M* solution (0.018 mole) of lithium aluminum hydride in ether was added dropwise over a period of 15 min. The mixture was allowed to warm to room temperature and stirred for 1 hr. The reaction mixture was filtered, and the blue filtrate was concentrated by evacuation at 0.01 mm for 2 hr. Crude N-thionitrosopiperidine (3.25 g) was obtained as a purple oil, $\lambda_{max}^{CCl_4}$ 585 mμ (ε 13) and $\lambda_{max}^{CCl_4}$ 318 mμ (ε 14,500). H^1 nmr spectrum shows a multiplet centered about τ 8.4 (6 H), a broad band centered at 6.2 (2 H), and a broad band centered at 5.7 (2 H). This sample was not obtained in analytical purity. Attempts to further purify this material by recrystallization or distillation resulted in decomposition.

N-Thionitrosopiperidine was also obtained from the reaction of sulfur with N-aminopiperidine by a procedure similar to that described for the preparation of N-thionitrosodimethylamine from dimethylhydrazine and sulfur.

N-Thionitrosohomopiperidine. This compound was prepared in crude form by the reaction of N-aminohomopiperidine with sulfur in ether. It was obtained as a purple oil, λ_{max}^{ether} 700, 584, and 306 mμ.

Reduction of N-Thionitrosodimethylamine. A 1 *M* solution of lithium aluminum hydride was added dropwise to a stirred solution of 0.25 g of N-thionitrosodimethylamine in 10 ml of ether cooled to 0° with an ice bath. The addition was stopped when the purple color faded. A few drops of water was cautiously added to decompose the excess hydride, and then 10 ml of 30% sodium hydroxide was added. The ether layer was separated, and the aqueous layer was extracted twice with 5 ml of ether. The ether solutions were combined and dried over powdered potassium hydroxide. The ether was decanted, and a few drops of a saturated ethereal solution of *p*-nitrophenyl isocyanate was added. The white precipitate that formed upon cooling was collected on a filter and recrystallized from alcohol. There was obtained 0.108 g of 1,1-dimethyl-4-*p*-nitrophenylsemicarbazide as light yellow crystals, mp 204–205°. An identical sample was also prepared from authentic 1,1-dimethylhydrazine and *p*-nitrophenyl isocyanate.

Anal. Calcd for $C_9H_{12}N_4O_3$: C, 48.21; H, 5.40; N, 24.99. Found: C, 48.29; H, 5.55; N, 24.76.

Synthesis of Phosphatidylglycerol and Diphosphatidylglycerol[1,2]

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Contribution from the Department of Biochemistry, Philadelphia General Hospital, Philadelphia, Pennsylvania 19104. *Received February 28, 1966*

Abstract: 1′,3′-Di-O-(1,2-di-O-stearoyl-L-glycerol-3-phosphoryl)glycerol (IIa) has been synthesized and compared with ox heart cardiolipin. (1,2-Di-O-stearoyl-L-glycerol-3-phosphoryl)-3′-D-glycerol (IIIa), having the configuration of the naturally occurring phosphatidylglycerol, has been synthesized; previously other workers had synthesized only an alternative stereoisomer or a mixture of stereoisomers.

During studies in this laboratory of the effect of X-irradiation on phospholipids of rat liver,[3] a particularly noticeable effect was observed in the behavior exhibited by phosphatidylglycerol. This observation and other recent work here has prompted our interest in the interrelation of phosphatidylglycerol and cardiolipin and their metabolic roles. Certain other laboratories have reported interest in these topics. The above compounds IIa and IIIa have been synthesized as model substrates to aid in these investigations. It is expected that synthetic phosphatidylglycerol having the configuration of the natural material will be metabolically more active than other stereoisomers.

(1) This work was carried out under Contract NYO 1864-21 with the U. S. Atomic Energy Commission, and supported in part by National Institutes of Health Training Grant 5 TI GM 1116.

(2) "Phosphatidyl" is an abbreviation for (1,2-diacyl-L-glycerol-3-phosphoryl).

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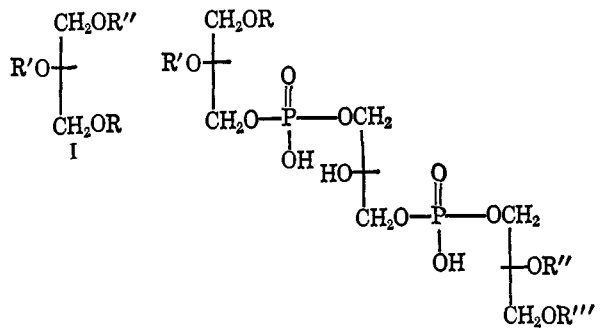
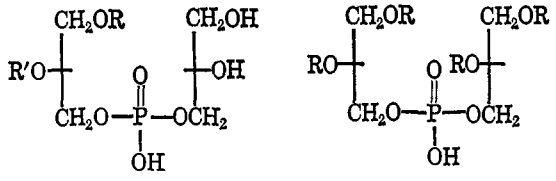


Ia, R = H; R′, R″ = stearoyl
b, R, R″ = H; R′ = benzyl
c, R′, R″ = H; R = benzyl
d, R′ = H; R = benzyl; R″ = trityl
e, R, R′ = benzyl; R″ = trityl
f, R″ = H; R, R′ = benzyl
g, R′, R″ = stearoyl; R = phosphate
IIa, R, R′, R″, R‴ = stearoyl
b, R, R′, R″, R‴ = long-chain fatty acyl residues



IIIa, R, R′ = stearoyl
b, R, R′ = long-chain fatty acyl residues

IVa, R = stearoyl

Diphosphatidylglycerol. In a recent publication synthesis of a diphosphatidylglycerol has been described,[4] the results providing evidence for a diphosphatidylglycerol structure (IIb) for ox heart cardiolipin.[5] Previously this structure has been assigned from hydrolysis observations.[6,7] In the course of our investigations we have synthesized a different diphosphatidylglycerol using a different procedure. Observations on this material also provide evidence for the diphosphatidylglycerol structure for ox heart cardiolipin. We should point out here that although this structure is correct for ox heart cardiolipin it does not appear to be correct for all cardiolipins.[8,9]

2,3-Di-O-stearoyl-D-glycerol (Ia) was prepared using published procedures with some modifications as described in the Experimental Section. A mixture of Ia and 2-O-benzylglycerol (Ib) prepared by a standard method was phosphorylated with phosphorus oxychloride. The reaction product was hydrogenated to remove the benzyl group and purified by silicic acid chromatography. Figure 1 illustrates the elution pattern. Thin layer chromatography (tlc) indicated peak I to be unreacted diglyceride Ia and the tailing portion peak III to be a mixture of phosphatidylglycerol and unidentified products. Peak II was identified as the diphosphatidylglycerol (IIa) and was crystallized as a sodium salt. It gave the expected elemental analyses and phosphorus:fatty acid:glycerol content ratios.

The ratios above are also the same as those generally accepted for ox heart cardiolipin.[6,10-12] Comparison

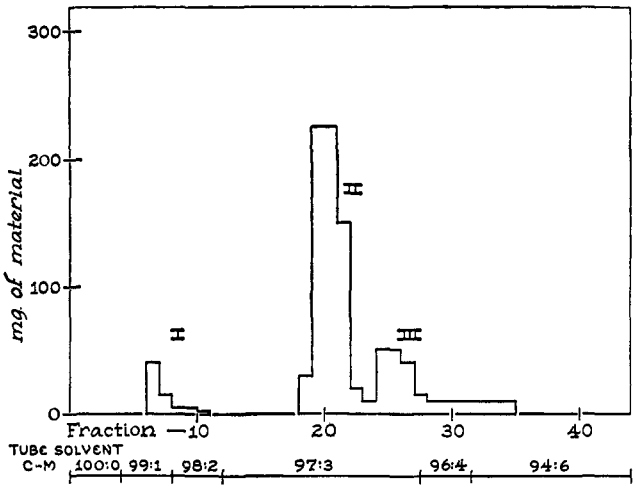


Figure 1. Column chromatography on silicic acid of the hydrogenated phosphorylation product in the synthesis of diphosphatidylglycerol. Solvents: C, chloroform; M, methanol.

of ox heart cardiolipin with the synthesized compound revealed little difference in optical rotation (natural cardiolipin $[\alpha]_D$ +5.5°; synthesized diphosphatidylglycerol $[\alpha]_D$ +5.0°) and in infrared absorption. Mild alkaline hydrolysis of the natural cardiolipin and of the synthetic material yielded products indistinguishable by paper ionophoresis and chromatography. These observations also provide evidence for the diphosphatidylglycerol structure assigned to ox heart cardiolipin.

Phosphatidylglycerol. Phosphatidylglycerol (IIIb) has been isolated from a variety of natural sources, but little is known concerning its metabolic role.[13] The configuration of the molecule was determined by its biosynthesis which showed it to have the structure phosphatidyl-3′-D-glycerol.[14] An alternative stereoisomer, phosphatidyl-3′-L-glycerol, has been synthesized,[15] and those workers engaged in the biosynthesis studies prepared a phosphatidyl-1′-DL-glycerol mixture. Synthesis of phosphatidylglycerol having the configuration of the naturally occurring material is described herein.

Tritylation of the well-known 3-O-benzyl-L-glycerol (Ic) yielded Id which was purified by chromatography on alumina. Benzylation of Id yielded Ie, the properties of which agreed with those reported for the same compound prepared several years ago.[16] Acid hydrolysis of Ie afforded the dibenzyl ether If. The optical rotation of the latter did not agree with that reported previously for the same compound,[16] the reason for which is not known. Phosphorylation with phosphorus oxychloride of a mixture of Ia and If followed by hydrogenation yielded a complex mixture which was purified by silicic acid chromatography. Figure 2 illustrates the elution pattern. Group analysis and tlc were employed to identify peak I as starting material Ia, peak II as bisphosphatidic acid (IVa), peak III as phosphatidic acid (Ig), and peak IV as the phosphatidylglycerol (IIIa). It was crystallized as a barium salt. This synthetic material gave good elemental

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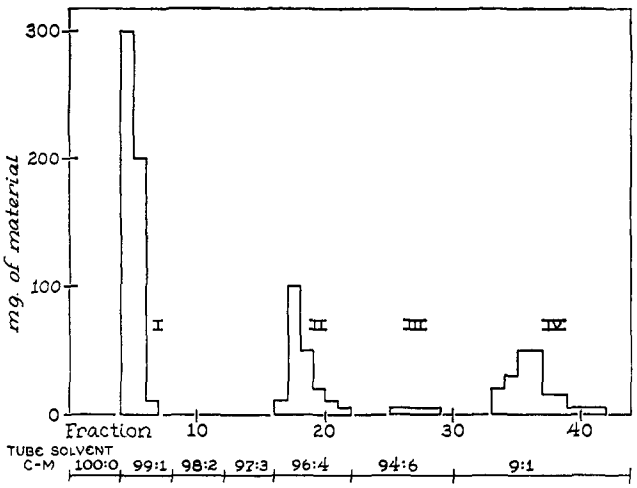

Figure 2. Column chromatography on silicic acid of the hydrogenated phosphorylation product in the synthesis of phosphatidylglycerol. Solvents: C, chloroform; M, methanol.

analyses and ratios close to those expected for phosphorus:fatty acid:glycerol content. Treatment with periodic acid[14] liberated approximately 1 mole of formaldehyde. On mild alkaline hydrolysis it yielded a product indistinguishable by paper chromatography and ionophoresis from a sample of glycerophosphorylglycerol.[17]

The synthesized phosphatidylglycerol was found to be very unstable when existing in the free acid form in solution, possibly due to isomerization and transesterification reactions of the phosphodiester group.[18] In view of this observation, qualitative oxidation of the terminal glycerol residue with periodic acid[14] should be viewed with caution.

Experimental Section

For column chromatography Merck reagent aluminum oxide and Mallinckrodt silicic acid (100 mesh) were used. The silicic acid was prewashed with chloroform, then methanol, and activated by heating at 110° for 1 hr. Merck silica gel H was used for tlc using the solvent system chloroform–methanol–water (65:25:4),[19] the components being detected with iodine vapor or by spraying with 50% sulfuric acid and charring. Paper chromatography and ionophoresis were carried out in the systems described for water-soluble phosphates,[20] the latter being detected with a modified Hanes–Isherwood spray.[21] Glycerol was determined by the method of Renkonen,[22] phosphorus by the method of Sperry,[23] and fatty acids by the method of Rapport and Alonzo.[24] Melting points were determined on a Thomas–Hoover block and are uncorrected. Ox heart cardiolipin was a commercial sample obtained from Nutritional Biochemicals Corp.

3-O-Benzyl-L-glycerol (Ic). 1-O-Benzyl-2,3-O-isopropylidene-D-glycerol (16.5 g, prepared by the method of Sowden and Fischer[25] with the modifications introduced by Howe and Malkin[26] from 2,3-O-isopropylidene-D-glycerol; the latter prepared from D-mannitol by the method of Baer and Fischer[27] with the modifications introduced by LeCocq and Ballou[6]) in 42 ml of 10% acetic acid was heated under reflux for 2 hr with stirring. The solution was cooled, saturated with sodium chloride, and exhaustively extracted with chloroform. The chloroform extract was dried and evaporated to yield a syrupy residue (12.6 g, 93%), $[\alpha]^{24}D$ +5.0° (*c* 10, chloroform) (lit.[25] $[\alpha]D$ +5.3°).

2,3-Di-O-stearoyl-D-glycerol (Ia). This was prepared from Ic *via* 1-O-benzyl-2,3-di-O-stearoyl-D-glycerol (mp 51°). It had mp 76° (lit. mp 71°,[26] 76–77°[28]).

2-O-Benzylglycerol (Ib). This was prepared according to Porck and Craig[29] *via* 2-O-benzyl-1,3-O-benzylideneglycerol (mp 78–80°). It had mp 39–40° (lit.[29] mp 37–39°).

1′,3′-Di-O-(1,2-di-O-stearoyl-L-glycerol-3-phosphoryl)glycerol (IIa). Compound Ia (2.49 g), dissolved in 6 ml of ether, 3 ml of alcohol-free chloroform, and 0.8 ml of pyridine, was added to 0.62 g of phosphorus oxychloride at 0°. The mixture was stirred at 0° for 1 hr, then 1 additional hr at room temperature. Ib (0.36 g), dissolved in 3.5 ml of alcohol-free chloroform and 0.4 ml of pyridine, was added and stirring was continued for 2 hr. Water (0.07 ml) was added and stirring was continued a further 30 min. Chloroform (150 ml) was added. The chloroform layer was washed with dilute sulfuric acid, saturated sodium carbonate solution, and finally water. The chloroform solution was dried and evaporated to dryness. The residue was dissolved in 75 ml of hexane and hydrogenated over a period of 24 hr using 0.6 g of palladium black catalyst. After filtration to remove the catalyst the solution was evaporated to dryness. The residue crystallized from acetone yielding 2.1 g of crystals. Part of these crystals (1.09 g) was dissolved in chloroform and chromatographed on 70 g of silicic acid. Fractions of 25 ml were collected. Chloroform–methanol (99:1 and 98:2) eluted Ia (0.075 g) identified by melting point and tlc; chloroform–methanol (97:3) first eluted the crystalline product IIa (0.75 g), then unidentified compounds, one of which migrated with phosphatidylglycerol on tlc. Increased concentrations of methanol gave additional unidentified material. The crystalline product was dissolved in chloroform–methanol (1:1) and neutralized with methanolic sodium hydroxide. It was recrystallized as a sodium salt from aqueous methanol, mp 90–92°, $[\alpha]^{24}D$ +5.0° (*c* 4.4, chloroform). The over-all yield was 50%. From an average of three determinations in each case it had phosphorus:fatty acid:glycerol content ratios of 2.0:3.92:3.02 (theoretical, 2:4:3).

Anal. Calcd for $C_{81}H_{155}O_{17}P_2Na_2$ (1507): C, 64.5; H, 10.3; P, 4.1. Found: C, 65.3; H, 10.2; P, 4.2.

1-O-Trityl-3-O-benzyl-L-glycerol (Id). Compound Ic (4 g) in 50 ml of pyridine was treated with 6.74 g of trityl chloride at room temperature for 40 hr. A syrupy residue was isolated by chloroform extraction. The syrup was dissolved in benzene and chromatographed on alumina. Benzene eluted triphenylcarbinol; benzene–ether (2:1) eluted the syrupy product (6.2 g, 67%), $[\alpha]^{26}D$ −3.6° (*c* 4, chloroform) (lit.[30] $[\alpha]D$ −4.5°).

1-O-Trityl-2,3-di-O-benzyl-L-glycerol (Ie). Compound Id (4.4 g) in 35 ml of benzyl chloride containing 8.3 g of powdered potassium hydroxide was heated at 110–120° for 3 hr with stirring. The cooled mixture was diluted with 150 ml of benzene. The benzene extract was washed with water, dried, and concentrated *in vacuo* up to a bath temperature of 135°. The remaining residue crystallized from methanol (3.75 g, 70%), mp 85°, $[\alpha]^{25}D$ −8° (*c* 2, ether) (lit.[16] mp 84.5–86°, $[\alpha]D$ −9°).

2,3-Di-O-benzyl-L-glycerol (If). This was prepared from Ie by the method of Wickberg.[16] It had $[\alpha]^{26}D$ +16° (*c* 2.6, chloroform). It was homogeneous by tlc; hydrogenation using palladium black catalyst yielded glycerol. Wickberg[16] reports $[\alpha]D$ +3.8°.

(1,2-Di-O-stearoyl-L-glycerol-3-phosphoryl)-3′-D-glycerol (IIIa). Compound Ia (2.37 g), dissolved in 6 ml of ether, 3 ml of alcohol-free chloroform, and 0.8 ml of pyridine, was added over a 10-min period to 0.58 g of phosphorus oxychloride at 0°. The mixture was stirred at 0° or 1 hr, then at room temperature for 1 hr. Compound If (1.03 g) dissolved in 3.5 ml of alcohol-free chloroform and 0.4 ml of pyridine was added. The mixture was stirred at room temperature for 2 hr. Water (0.07 ml) was added and stirring was continued for an additional 30 min. Ether (150 ml) was added. The ether layer was filtered to remove pyridine hydrochloride (1.1

(17) Glycerophosphorylglycerol was a specimen supplied by Dr. A. A. Benson. See A. A. Benson and B. Maruo, *Biochim. Biophys. Acta*, 27, 189 (1958).
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g, 84%), and washed with dilute sulfuric acid, saturated sodium carbonate solution, and water. After drying, evaporation to dryness yielded a syrupy residue (3.08 g). This residue (2.85 g) was dissolved in 30 ml of hexane and hydrogenated over a period of 24 hr using 0.6 g of palladium black catalyst. The solution was filtered and evaporated to dryness, and the residue was dissolved in 30 ml of hot, dry acetone. On cooling 1.65 g of crystals was deposited, mp 68°. Tlc indicated at least four components. The crystals (1 g) were dissolved in chloroform and chromatographed on 75 g of silicic acid. Fractions of 25 ml were collected. Chloroform–methanol (99:1) eluted Ia (0.51 g) identified by melting point and tlc. Chloroform–methanol (96:4) eluted material tentatively identified as bis(1,2-di-O-stearoyl-L-glycerol) phosphate (IVa, 0.18 g); it had mp 69–70° (lit.[31] mp 69.5–70.5°) and phosphorus:fatty acid:glycerol content ratios of 1.0:4.1:2.3 (theoretical, 1:4:2). Chloroform–methanol (94:6) eluted material tentatively identified as 1,2-di-O-stearoyl-L-glycerol 3-phosphate (Ig, 0.022 g); it had phosphorus:fatty acid:glycerol content ratios of 1.0:2.0:1.2 (theoretical, 1:2:1). Chloroform–methanol (9:1) eluted the product IIIa (0.19 g). It was dissolved in warm acetone and neutralized with barium hydroxide. On cooling, crystals were deposited, mp 166°, $[\alpha]^{25}D$ +9.2° (*c* 1, pyridine). The over-all yield was 11%. From an average of three determinations in each case it had phosphorus:fatty acid:glycerol content ratios of 1.0:1.9:1.9 (theoretical, 1:2:2). The yield of formaldehyde released per mole of material oxidized with periodic acid[14] was 0.86, Ic being employed as a standard.

Anal. Calcd for $C_{42}H_{82}O_{10}P_1Ba_{0.5}$ (845): C, 59.6; H, 9.7; P, 3.7. Found: C, 59.0; H, 10.0; P, 3.6.

Alkaline Hydrolysis. The synthetic IIa and IIIa and ox heart cardiolipin were deacylated by the method described by Dawson and co-workers[20] for the identification of phospholipids in biological specimens. Samples from the three hydrolysates were subjected to paper chromatography and ionophoresis as described by the same workers. The hydrolysates from IIa and ox heart cardiolipin were identical in behavior. The hydrolysate from IIIa gave an identical picture with that of glycerophosphorylglycerol.[17]

Acknowledgment. We wish to thank Dr. A. A. Benson for a generous gift of glycerophosphorylglycerol, and Miss Lorraine Dreisbach for excellent technical assistance.

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7-D-Proline-oxytocin and Its Deamino Analog. Diastereoisomers of Oxytocin and Deamino-oxytocin[1]

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Contribution from the Department of Biochemistry, Cornell University Medical College, New York, New York 10021. *Received April 20, 1966*

Abstract: 7-D-Proline-oxytocin, an analog differing from the posterior pituitary hormone only in the configuration of the amino acid residue in the 7 position, has been synthesized and tested for oxytocic and avian vasodepressor activities. The required synthetic nonapeptide intermediate was prepared from D-prolyl-L-leucylglycinamide by the stepwise *p*-nitrophenyl ester method. Reduction with sodium in liquid ammonia and subsequent oxidation of the sulfhydryl form to the cyclic disulfide yielded 7-D-proline-oxytocin. The analog exhibited 13 units/mg of oxytocic activity but the avian vasodepressor was nil. 1-Deamino-7-D-proline-oxytocin was also synthesized and found to possess 45 units/mg of oxytocic activity and no avian vasodepressor activity. For the preparation of D-prolyl-L-leucylglycinamide, DL-proline was used as the starting material. Resolution of the diasteroisomeric tripeptides resulting from the reaction of *p*-nitrophenyl carbobenzoxy-DL-prolinate with ethyl L-leucylglycinate was accomplished and the ethyl carbobenzoxy-D-prolyl-L-leucyglycinate so obtained was converted to the amide.

As a further contribution to the study of the relationship of the stereostructure of oxytocin to manifestation of pharmacological activity, 7-D-proline-oxytocin has been synthesized. This diastereoisomer of oxytocin differs from oxytocin (Figure 1) only in the configuration of the proline residue in the 7 position. The required intermediate nonapeptide for the synthesis of 7-D-proline-oxytocin, namely N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-D-prolyl-L-leucylglycinamide, was obtained by the stepwide *p*-nitrophenyl ester method employed by Bodanszky and du Vigneaud for the synthesis of oxytocin,[2] but with D-prolyl-L-leucylglycinamide used as the starting material.

At the time the synthesis was undertaken D-proline was not available commercially. For the preparation of the desired ethyl D-prolyl-L-leucylglycinate intermediate the diastereoisomeric tripeptides resulting from the reaction of *p*-nitrophenyl carbobenzoxy-DL-prolinate with ethyl L-leucylglycinate were separated by fractional crystallization and thus the resolution of the DL-proline and the preparation of the desired tripeptide ester was accomplished in one step.

The ethyl D-prolyl-L-leucylglycinate was then converted to the amide and used for the subsequent synthetic steps for the preparation of the protected nonapeptide. The latter compound was treated with sodium in liquid ammonia to remove the protecting groups by the method of Sifferd and du Vigneaud[3] as used in the original synthesis of oxytocin.[4] The dithiol so obtained was oxidized to the cyclic disulfide by aeration in aqueous solution at pH 6.8. Purification of the D-proline analog was accomplished by countercurrent distribution[5] and by partition chromatography on Sephadex

(1) This work was supported in part by Grant HE-01675 from the National Heart Institute, U. S. Public Health Service.

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