602. *A New Synthesis of Phosphatidylserine and the Preparation of Serine and 2-Aminoethyl ("Ethanolamine") Phosphate Esters.*

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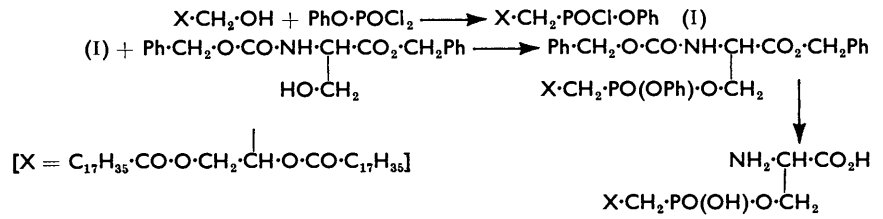
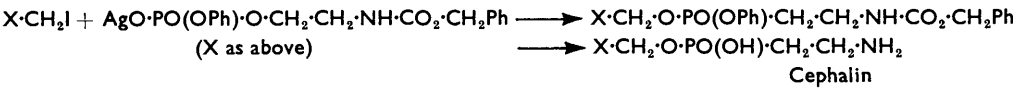
Phosphatidylserine has been prepared in good yield by the interaction of glycerol 1-iodide 2 : 3-distearate and *N*-benzyloxycarbonyl-DL-serine benzyl ester 3-(silver phenyl phosphate) in boiling xylene in the dark, followed by catalytic hydrogenolysis. Serine and 2-aminoethyl (ethanolamine) *O*-phosphate are prepared in high yield by the action of diphenyl phosphorochloridate on DL-*N*-benzyloxycarbonylserine benzyl ester and *N*-benzyloxycarbonylethanolamine, respectively, followed by catalytic hydrogenolysis.

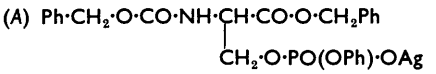
Phosphatidylserine was discovered in ox-brain phospholipids by Folch,[1] who showed it to be an oleoylstearoylphosphatidyl ester of L-serine. Baer and Maurukas[2] then established the detailed structure by the synthesis of *O*-(L-1 : 2-distearoylphosphatidyl)-L-serine (I), identical with the reduction product of phosphatidylserine from ox brain. Baer and Maurukas's synthesis, outlined below, is a general synthesis for phosphatidyl compounds but has disadvantages. *E.g.*, 1 : 2-diglycerides, the starting materials, are difficult to prepare[3] and change readily by acyl migration into the 1 : 3-isomers; and the phosphorylation stage gives diphosphatidyl compounds which are not easily removed.



In order to overcome these difficulties in the related syntheses of cephalin[4] and plasmalogen,[5] Malkin and his collaborators used silver 2-*N*-benzyloxycarbonylaminoethyl phenyl phosphate, which leads to high yields :



This method has now been extended to the synthesis of phosphatidylserine. Glycerol 1-iodide 2 : 3-distearate was allowed to react with the *N*-benzyloxycarbonyl-DL-serine benzyl ester 3-(silver phenyl phosphate) (*A*) in boiling xylene in the dark, and the protecting groups were then removed by catalytic hydrogenolysis. The yield based on diacyl iodide was 62%; Baer and Maurukas obtained 23%, based on diglyceride.



So far we have prepared only DL-compounds by this method, but its adaptation to L-compounds is under investigation.

[1] Folch, *J. Biol. Chem.*, 1941, **139**, 973; 1942, **146**, 35; 1948, **174**, 439.
[2] Baer and Maurukas, *ibid.*, 1954, **212**, 25.
[3] Cf. Howe and Malkin, *J.*, 1951, 2663.
[4] Baylis, Bevan, and Malkin, Report on Biochemical Problems of Lipids, Ghent (Butterworths, London, 1955), p. 91.
[5] Malkin, Baylis, Bevan, and Webley, *Olii, Grassi, Colori*, 1956, **33**, 226.

Serine 3-(dihydrogen phosphate) has been prepared by Levene and Schormüller [6] by the action of phosphoric acid and phosphorus on serine and of phosphorus oxychloride on benzylideneserine, and Plimmer [7] used the former method under pressure. Ethanolamine phosphate (2-aminoethyl dihydrogen phosphate) has been prepared by Outhouse,[8] Plimmer and Burch,[9] Christensen,[10] and Cherbuliez and Weniger,[11] but all their methods give poor yields or impure products; we have prepared both phosphates in high yield by the action of diphenyl phosphorochloridate on DL-*N*-benzyloxycarbonylserine benzyl ester and *N*-benzyloxycarbonylethanolamine, respectively, followed by catalytic hydrogenolysis of the protecting groups in acetic acid, with equal amounts of palladium black and Adams catalyst.

After the work on ethanolamine phosphate was completed, Baer and Stancer [12] reported an identical synthesis except that the hydrogenolysis was carried out in two stages. The first was carried out in alcohol, with palladium, and the reaction was then completed by further reduction in acetic acid with Adams catalyst. We had noted that ethanolamine *O*-(phenyl hydrogen phosphate) separated almost completely from solution in alcohol with either catalyst and this is the cause of the incomplete hydrogenolysis reported by Baer and Stancer.

Experimental

N-*Benzyloxycarbonyl*-DL-*serine Benzyl Ester*.—A mixture of *N*-benzyloxycarbonyl-DL-serine [2] (4·8 g.), dry benzyl alcohol (25 c.c.), and toluene-*p*-sulphonic acid (0·3 g.) was disilled under reduced pressure (water-pump) from a bath at $\geqslant 130°$. After the bulk of the benzyl alcohol had distilled off, a further 25 c.c. of benzyl alcohol were added and distilled off in the same manner. The residue was treated in ether (50 c.c.) with saturated sodium hydrogen carbonate solution (50 c.c.), washed with water (2 × 50 c.c.), and dried. Removal of ether and crystallisation from 5 : 2 carbon tetrachloride–light petroleum (b. p. 40—60°) gave pure benzyl ester (4·5 g., 68%), m. p. 72·5°. Baer and Maurukas [2] used benzyl bromide which our method avoids.

N-*Benzyloxycarbonyl*-DL-*serine Benzyl Ester* 3-(*Potassium Phenyl Phosphate*).—To a mixture of phenyl phosphorodichloridate (4·3 g., 0·02 mole) and quinoline (2·6 g., 0·02 mole) in dry chloroform (15 c.c.), was added *N*-benzyloxycarbonyl-DL-serine benzyl ester (6·6 g., 0·02 mole) in dry chloroform (40 c.c.), with ice-cooling and stirring, during 3 hr. After a further ½ hour's stirring at 0°, and 2 hours' at room temperature, pyridine (2 c.c.) and water (1 c.c.) were added, and stirring was continued for ½ hr. The solvent was then removed under reduced pressure at $<40°$, and the residue taken up in water (15 c.c.). Addition of potassium carbonate (4·2 g., 3 equivs.) precipitated the *potassium salt* of the phosphate, and after extraction of the suspension with ether (3 × 25 c.c.) to remove quinoline and impurities, the salt was filtered off. A further small amount was obtained from the filtrate on slight evaporation and storage. The white micro-crystalline powder, crystallised from ethanol, had m. p. 184·5° (7·3 g., 70%) (Found : C, 55·4; H, 4·55; N, 2·6; P, 5·9. $C_{24}H_{23}O_8NPK$ requires C, 55·1; H, 4·4; N, 2·7; P, 5·9%).

The Silver Salt of N-*Benzyloxycarbonyl*-DL-*serine Benzyl Ester* 3-(*Silver Phenyl Phosphate*).—The above potassium salt (1·2 g.) was dissolved in hot water (5 c.c.), and to the cooled solution was added silver nitrate (0·5 g.) in the minimum amount of water. A viscous product separated, and after decantation of the aqueous layer, was triturated with a little water which was then decanted, and the process repeated. The viscous *salt*, on drying over P_2O_5 in *vacuo*, became a glass which ground to a white powder (1·3 g., 95·7%) (Found : Ag, 17·9. $C_{24}H_{23}O_8NPAg$ requires Ag, 18·2%). This material softened at 40° and gave a meniscus at 95°.

The preparation was carried out with the minimum exposure of the product to light.

O-(1 : 2-*Distearylphosphatidyl*)*serine*.—To the above silver salt (0·9 g.) in boiling dry xylene (15 c.c.) was added glycerol 1-iodide 2 : 3-distearate (0·9 g.), and the mixture was refluxed and stirred in the dark for 15 min. with exclusion of moisture. After cooling, silver salts were

[6] Levene and Schormüller, *J. Biol. Chem.*, 1934, **105**, 547; **106**, 595.
[7] Plimmer, *Biochem. J.*, 1941, **35**, 461.
[8] Outhouse, *ibid.*, 1936, **30**, 199; 1937, **31**, 1547.
[9] Plimmer and Burch, *ibid.*, 1937, **31**, 398.
[10] Christensen, *J. Biol. Chem.*, 1940, **135**, 399.
[11] Cherbuliez and Weniger, *Helv. Chim. Acta*, 1946, **29**, 2006.
[12] Baer and Stancer, *Canad. J. Chem.* 1956, **34**, 436.

removed by filtration with Filter Cel and the filtrate was evaporated to dryness under reduced pressure at <40°. The residue was dissolved in ether and washed twice with sodium hydrogen carbonate solution, then with water, dried, and evaporated to dryness, to yield 1·2 g. of solid material. This was hydrogenated in acetic acid (50 c.c.) at atmospheric pressure and 40°, in the presence of 1 : 1 platinum dioxide–palladium (2 g.), the palladium having been washed free from alkali as previously described.[13] When hydrogen ceased to be taken up, excess of hydrogen was removed by evacuation, chloroform added to dissolve precipitated organic material, and the catalyst removed by filtration with Filter Cel. The filtrate was evaporated to dryness under reduced pressure and the residue extracted with boiling ether (3 × 25 c.c.), the product being separated each time in a centrifuge. This product was dissolved in warm chloroform, and the solution was filtered (with Filter Cel) and evaporated to dryness under reduced pressure at <40°. The white *serine derivative* (0·6 g., 61·8% calc. on iodide distearate) melted at 162° (decomp. after slight sintering at 157°) (Found : C, 63·4; H, 10·5; N, 1·9; P, 3·7. Calc. for $C_{42}H_{82}O_{10}NP$: C, 63·7; H, 10·4; N, 1·77; P, 3·9%).

The ethereal extracts deposited small amounts of 1 : 3-distearin.

DL-*Serine* 3-(*Dihydrogen Phosphate*).—*N*-Benzyloxycarbonyl-DL-serine benzyl ester (1·65 g.) was added slowly with shaking to diphenyl phosphorochloridate (1·47 g., 10% excess) in dry pyridine (7 c.c.). It is important to avoid rise in temperature at this stage. Next morning a few drops of water were added to decompose excess of phosphorochloridate, and after an hour, the product was poured into water, and the precipitated viscous oil was extracted with ether. The ethereal solution was washed with dilute sulphuric acid, 5% sodium hydrogen carbonate solution, and water, dried (Na_2SO_4), and evaporated to dryness under reduced pressure at 40°. The resulting viscous *phosphate* weighed 2·52 g. (90%) (Found : C, 64·0; H, 5·2; N, 2·9; P, 5·4. $C_{30}H_{28}O_8NP$ requires C, 64·2; H, 5·0; N, 2·5; P, 5·5%).

Hydrogenolysis. The oil (2·4 g.) was hydrogenated in acetic acid (30 c.c.) with the platinum oxide–palladium (2 g.) described above. After 700 c.c. of hydrogen had been absorbed, uptake of hydrogen became very slow, and water (30 c.c.) was added to dissolve crystalline material which had separated. Hydrogenation recommenced and ceased after a further 400 c.c. had been absorbed (theor. 958 c.c.). The catalyst was removed and the filtrate was evaporated to dryness under reduced pressure at <40°. The resultant white solid was difficult to crystallise and this was best effected by dissolving it in the minimum amount of water and adding ethanol dropwise. The yield was 0·64 g. (80%) and the m. p. 164—165° (Found : C, 19·6; H, 4·4; N, 7·6; P, 16·5. Calc. for $C_3H_8O_6NP$: C, 19·5; H, 4·3; N, 7·6; P, 16·8%). The crystalline material which separated during the first stage of the above hydrogenolysis was the monophenyl ester. In one experiment, the mixture was filtered before the addition of water, and a yield of 70% of this intermediate was obtained by extracting the precipitate with water, and evaporating the aqueous solution to dryness under reduced pressure at <40°. DL-*Serine* 3-(*phenyl hydrogen phosphate*) forms white crystals, m. p. 167—168°, when dissolved in the minimum amount of water and treated with ethanol dropwise (Found : C, 40·8; H, 4·6; N, 5·5; P, 11·7. $C_9H_{12}O_6NP$ requires C, 41·4; H, 4·6; N, 5·6; P, 11·9%). The low carbon content is probably due to the presence of a trace of dihydrogen phosphate (C, 19·5%).

Ethanolamine Phosphate (2-*Aminoethyl Dihydrogen Phosphate*).—Finely powdered *N*-benzyloxycarbonylethanolamine [14] (3 g.) was added in small portions to diphenyl phosphorochloridate (4·5 g., 10% excess) in dry pyridine (15 c.c.) with shaking and cooling to avoid local temperature rise. After being kept overnight, the product was worked up as for serine phosphate, yielding 6·3 g. (96%) of viscous oil (Found : C, 61·6; H, 5·2; N, 3·6; P, 7·0. Calc. for $C_{22}H_{22}O_6NP$: C, 61·8; H, 5·4; N, 3·3; P, 7·3%).

Hydrogenolysis. This oil (5 g.) was hydrogenated in acetic acid (30 c.c.) at atmospheric pressure with the above catalyst (2·5 g.), used in portions of 1 g., 1 g., and 0·5 g. added at intervals, as the absorption of hydrogen tended to slacken. After 8 hr., 3 l. had been absorbed and the mixture was filtered. Precipitated ethanolamine phosphate was extracted from the catalyst with water, and the acid filtrate and the aqueous extract were combined and evaporated to dryness under reduced pressure at 40°. The residue crystallised on dissolution in a small amount of water and precipitation by ethanol (yield 1·5 g.; m. p. 242·3°) (Found : C, 16·7; H, 5·5; N, 9·7; P, 21·7. Calc. for $C_2H_8O_4NP$: C, 17·0; H, 5·7; N, 9·9; P, 22·0%).

Hydrogenolysis in ethanol gave a crystalline precipitate and absorption of hydrogen ceased

[13] Brown, Malkin, and Maliphant, *J.*, 1955, 1584.
[14] Rose, *J. Amer. Chem. Soc.*, 1947, **69**, 1384.

after about 2 hr. The precipitate was filtered off and extracted from the catalyst with water. After evaporation to small bulk under reduced pressure, it crystallised on addition of ethanol. It melts at 252—254° and is ethanolamine *O*-(phenyl hydrogen phosphate) (Found : C, 43·8; H, 5·7; N, 6·2; P, 14·3. $C_8H_{12}O_4NP$ requires C, 44·2; H, 5·5; N, 6·5; P, 14·5%).

Ethanolamine diphenyl phosphate is found in the alcoholic mother-liquor of the above experiment and has m. p. 132—133° [from alcohol–light petroleum (b. p. 40—60°). The p-*nitrobenzoyl derivative* melted at 113° (from ethanol) (Found : C, 56·9; H, 4·5; N, 6·3. $C_{21}H_{19}O_7N_2P$ requires C, 57·0; H, 4·3; N, 6·3%).

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