the mixture refluxed 2 hr. Cooling, evaporation to half volume, and addition of 200 ml of water produced a solid. Filtration and thorough washing with water and ether yielded analytically pure material which did not melt below 340°.

Many of these compounds form tenacious hydrates and were dried for analysis at 132° under vacuum over P_2O_5 for 48 hr. Certain compounds (especially 2-methyl analogs) retained water of hydration even under these drying conditions.

B.—After attempting method A and detecting incomplete reaction by thin layer chromatography, the reaction mixture was heated at 125° for 17–20 hr and then hydrolyzed and purified as above.

C.—This method was similar to method A except that the reactants were combined in a pressure bottle and heated for 18 hr at 125°.

Acknowledgment.—The author is grateful to Dr. Stuart Paulson for carrying out the biological testing.

Total Synthesis of Unsaturated Phosphatidylserine Using the N-4-Chlorobutyroyl Protecting Group[1]

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Large amounts of unsaturated phosphatidylserines with defined fatty acid composition are needed for the systematic study of the effect of phospholipids on hemostasis, and these phosphatidylserines can only be obtained by total synthesis. The available syntheses have suffered in yield from the relatively energetic methods used for the removal of protecting groups.

Recently, Eschenmoser's group[2] has introduced an easily removable protecting group, the 4-chlorobutyroyl group, and we decided to explore the applicability of this group in phosphatide synthesis.

Experimental Section[3]

N-(4-Chlorobutyroyl)-O-benzyl-(±)-serine methyl ester was prepared exactly as described for the corresponding tryptophan derivative,[2] replacing the tryptophan methyl ester hydrochloride with O-benzyl-(±)-serine methyl ester hydrochloride.[4] The product was crystallized from CCl_4–hexane, mp 42°, yield 84%.

Anal. Calcd for $C_{15}H_{20}ClNO_4$: C, 57.41; H, 6.43; N, 4.47. Found: C, 57.39; H, 6.54; N, 4.60.

N-(4-Chlorobutyroyl)-O-benzyl-(±)-serine. A. By Hydrolysis of Methyl Ester.—The methyl ester (20 g) was dissolved in 60 ml of acetone and to this was added 1 *N* NaOH dropwise as the alkali was consumed. The hydrolysis was slow at first but speeded up as part of the alkali was consumed. When close to the theoretical amount of alkali was used up, 100 ml of water was added and crystals of O-benzyl-(±)-serine were filtered out. The mother liquor was cooled and acidified with ice-cold 1 *N* phosphoric acid, giving an oil which solidified to 8.2 g (43%) of crystals, mp 117°, identical by infrared spectrum and mixture melting point with material obtained by method B below.

B. By Ronwin[5] Acylation of Acid.—Dry O-benzyl-(±)-serine[4] (48.5 g, 0.25 mole) was refluxed and stirred with 28 ml (0.25 mole) of 4-chlorobutyroyl chloride in 2 l. of dry ethyl acetate for 6 hr. The solution was filtered and evaporated *in vacuo* to a small volume. Addition of CCl_4 gave 42 g of the acylated material (57%). Recrystallized from ethyl acetate–petroleum ether, it had mp 120°.

Anal. Calcd for $C_{14}H_{18}ClNO_4$: C, 56.09; H, 6.05. Found: C, 56.27; H, 6.04.

Phthalimidomethyl Ester of O-Benzyl-N-(4-chlorobutyroyl)-(±)-serine.—A solution of 3 g (0.01 mole) of the preceding acylated O-benzylserine in 20 ml of dry DMF was treated with 1.97 ml (0.01 mole) of dicyclohexylamine and a solution of 1.95 g (0.01 mole) of phthalimidomethyl chloride in 20 ml of dry DMF. The mixture was kept at 37° overnight. It was diluted with water and allowed to crystallize. Recrystallized from ethanol the collected crystals had mp 125°, yield 2.8 g.

Anal. Calcd for $C_{23}H_{23}ClN_2O_6$: C, 60.19; H, 5.05; N, 6.11. Found: C, 60.44; H, 5.24; N, 5.89.

Phthalimidomethyl Ester of N-(4-Chlorobutyroyl)-(±)-serine.—The benzyl ester (10 g) was suspended in peroxide-free tetrahydrofuran (THF) and hydrogenated at 3 atm over the Pd catalyst of Tausz and Von Putnoky.[6] After the addition of boiling acetone to dissolve precipitated product, the catalyst was filtered, and the solution was concentrated in part and cooled to give crystals, mp 145°, yield 5 g. This hydrogenation did not always proceed as smoothly as described and the product gave a test with ninhydrin indicating an N → O shift. Such material was purified by chromatography on silica gel (British Drug Houses, Inc.) by eluting the desired product with acetone. The purity of the material was conveniently investigated by tlc using the solvent system *n*-butyl ether–propionic acid–water (120:90:11) with silica gel H (E. Merck, Darmstadt) and the staining procedure of Pataki.[7]

Anal. Calcd for $C_{16}H_{17}ClN_2O_6$: C, 52.11; H, 4.65; N, 7.60. Found: C, 51.84; H, 4.53; M, 7.49.

DL-Phosphatidyl-(oleoyl stearoyl)-DL-serine.[8]—1-Oleoyl-2-stearoylglycerol[10] (9 g) was brought into reaction with equivalent quantities of $POCl_3$ and the protected serine in the presence of pyridine and quinoline under standard conditions.[9,11] The crude product was extracted with anhydrous ether and with hexane, and the material insoluble in these two solvents was rejected. The phosphatide appeared to be in the soluble portion by infrared spectrum. This portion weighed 7 g. The material was dissolved in 75 ml of peroxide-free THF (distilled over excess triphenylphosphine) and to the solution was added a mixture of 48 ml of 0.5 *N* aqueous silver perchlorate and 75 ml of water. The mixture was shaken mechanically under nitrogen in the dark for 40 hr. The THF was removed by distillation and the aqueous layer was extracted ($CHCl_3$). The $CHCl_3$ solution was washed with dilute KCl solution, dried, and distilled finally in a high vacuum. The dry residue was dissolved in 90 ml of ethanol and cooled in an ice bath. To the solution was added 8.4 m*M* hydrazine in 90 ml of ethanol. The reaction mixture was stored at room temperature for 30 min and then evaporated.[12] The crude product was purified by the usual techniques of chromatography on silicic acid and DEAE-cellulose (acetate form).[9] The yield was 727 mg of crude phosphatidylserine (one chromatogram) of which half could be recovered as completely homogeneous material after a second chromatograph. The product showed only one spot moving opposite to natural phosphatidylserine when chromatographed in amounts of 250 μg on silicic acid impregnated paper; it had the infrared spectrum of phos-

(1) Supported by Grant No. A-533 of the U. S. Public Health Service.

(2) H. Peter, M. Brugger, J. Schreiber, and A. Eschenmoser, *Helv. Chim. Acta*, **46**, 577 (1963).

(3) Solvents were dried over Molecular Sieve 4A of Linde Products Division, Union Carbide Corp. 4-Chlorobutyroyl chloride was purchased from Aldrich Chemical Co., Inc., and was used as received. Oleic acid was 99% purchased from Applied Science Laboratories, Inc., State College, Pa. Stearic acid 99% was purchased from Stearinerie Dubois Fils, Usine de Scoury (Indre), France. Melting points were determined on the Kofler bar and infrared spectra on the Perkin-Elmer Infracord spectrophotometer. Evaporations were done *in vacuo* in a rotating evaporator. All operations with unsaturated material were under oxygen-free nitrogen.

(4) W. Grassmann, E. Wünsch, P. Deufel, and A. Zwick, *Chem. Ber.*, **91**, 538 (1958).

(5) *Cf.* (a) E. Ronwin, *J. Org. Chem.*, **18**, 127 (1953); (b) *ibid.*, **18**, 1546 (1953); (c) *ibid.*, **22**, 1180 (1957); (d) E. Ronwin and C. B. Warren, *ibid.*, **29**, 2276 (1964).

(6) J. Tausz and N. Von Putnoky, *Chem. Ber.*, **52**, 1573 (1919).

(7) G. Pataki, *J. Chromatog.*, **12**, 541 (1963).

(8) For nomenclature see ref 9, footnote 1.

(9) D. L. Turner, M. J. Silver, E. Baczynski, N. Giordano, and I. Rodalewicz, *J. Lipid Res.*, **5**, 616 (1964).

(10) N. A. Bogoslovskii, G. I. Samoshvalov, and N. A. Preobrazhenskii, *Zh. Obshch. Khim.*, **31**, 1143 (1961).

(11) E. Baer and D. Buchnea, *J. Am. Chem. Soc.*, **81**, 1758 (1959).

(12) The reaction period was reduced because the more usual conditions[9,11] gave poorer yields, possibly connected with the presence of colloidal silver.

phatidylserine. Analysis of the fatty acid composition by glc showed the expected composition of an equal amount of stearic and oleic acid.

Anal. Calcd for $C_{42}H_{80}NO_{10}P$: C, 63.85; H, 10.21; N, 1.77; P, 3.92. Found: C, 64.04; H, 10.02; N, 1.83; P, 3.76.

Biological Results.—The phosphatidyl-(oleoyl stearoyl)-serine was suspended in buffered saline solution or solubilized with buffered sodium deoxycholate solution and tested in several tests of blood coagulation described earlier.[9,13] The results indicated that the material had the same quantitative activity as the phosphatidyl-(oleoyl stearoyl)-serine prepared earlier[9] by a different method, *i.e.*, weak to moderate acceleratory activity in simple suspensions, and anticoagulant activity when well solubilized. This activity was present although the material was racemic.

Acknowledgment.—Analysis of the fatty acids from the phosphatidylserine was kindly performed for us by S. F. Herb and Francis E. Luddy of the Eastern Regional Research Laboratory, U. S. Department of Agriculture, Wyndmoor, Pa., using the techniques developed by them. References to the publications describing their techniques can be found in our earlier paper.[9] We wish to thank Mrs. Peggy Moser for technical assistance.

(13) M. J. Silver, D. L. Turner, I. Rodalewicz, N. Giordano, R. Holburn S. F. Herb, and F. E. Luddy, *Thromb. Diath. Haemorrhag.*, **10**, 164 (1963).

1,3,2-Diazaphosphorine 2-Oxides. III.[1] Preparation and Biological Evaluation of Some 1,3-Bis(aralkyl)-2-halo-1,3,2-diazaphosphorine 2-Oxides and Related Compounds[2]

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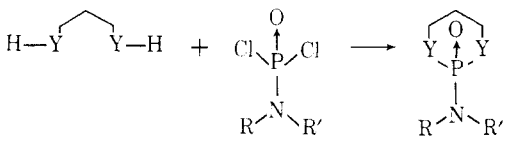
The discovery of 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide (I)[3] as a valuable antitumor drug has stimulated considerable interest in derivatives of phosphoric acid.[4] The standard procedure for the preparation of I and other closely related heterocyclic ring systems of the same general type has been by the reaction of diamines, diols, and amino alcohols with phosphoramidic dichloride according to Scheme I.[5]

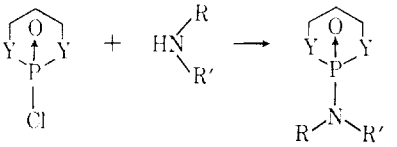
SCHEME I



Y = O, NH

Although Scheme I is in general quite satisfactory, it is limited in its scope due to the fact that phosphoramidic dichlorides cannot be prepared from amines containing other functional groups which are sensitive to phosphorus oxychloride. In order to overcome this difficulty, an alternate method for synthesizing I and related heterocyclic molecules would be to use chlorodiazaphosphorine 2-oxides (II), chlorodioxaphosphorinane 2-oxides (III), or chlorooxazaphosphorines (IV) and allow them to react with the appropriate amine. If the latter type chlorides were available, this would also afford the opportunity for the preparation of many new amides (Scheme II) that may be active antitumor agents.

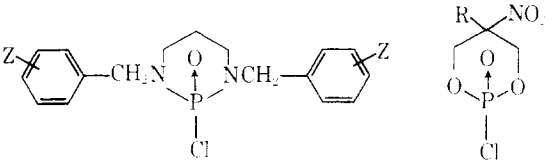
SCHEME II



Y = O, NH

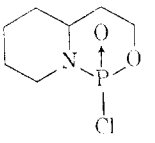
A review of the literature reveals little information concerning the preparation of chlorophosphorine 2-oxides. It was therefore decided that a study should be made to determine if a good general method could be developed for making such chlorides.

The present investigation reveals that the chlorophosphorine 2-oxides II and IV and the chlorophosphorinane III can be made in very satisfactory yields (Table I) by the general reaction shown in Scheme III.



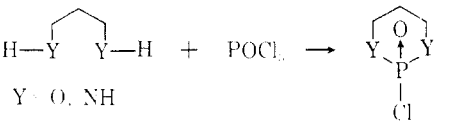
IIa, Z = *p*-Cl
b, Z = *p*-OCH_3
c, Z = *p*-$N(CH_3)_2$

IIIa, R = CH
b, R = C_2H_5



IV

SCHEME III



Y = O, NH

Compounds of type II were the first to be synthesized in the hope of finding a more versatile route to the 1,3-bis(aralkyl)-2-(N-arylamino)-1,3,2-diazaphosphorine 2-oxides (VIII) which have been of interest as potential antitumor agents in this laboratory.[1] The original method used for making these latter compounds involved the reaction of a diamine (VI) with a phosphoramidic dichloride (VII), as shown below in Scheme IV in the equation labeled path A. Now that compounds of type II are available, it is anticipated that the same products VIII can be synthesized according to path B.

(1) Part II: J. H. Billman and J. L. Meisenheimer, *J. Med. Chem.*, **8**, 264 (1965).

(2) This investigation was supported by a Public Health Service Grant (CA-06448-03) from the National Institutes of Health, Public Health Service.

(3) Cytoxan®.

(4) H. Arnold, F. Bourseaux, and N. Brock, *Arzneimittel-Forsch.*, **11**, 146 (1961).

(5) H. Arnold and F. Bourseaux, *Angew. Chem.*, **70**, 539 (1938).