carbon-hydrogen analysis of each product are given in Table II and the infrared results are compiled in Table III.

TABLE III

PERCENTAGE OF *cis* ISOMER PRODUCED

	Run 1		Run 2		Run 3		Run 4	
	11.7 μ	12.3 μ	11.7 μ	12.3 μ	11.7 μ	12.3 μ	11.7 μ	12.3 μ
IXb	39	37	36	37	33	34	37	37
IXa	60	57	56	54	62	58	51	54
Xb	44	38	47	39	49	44	46	39
Xa	45	37	48	40	56	42	..	..

4-Phenylmercaptocamphane (XI).—4-Camphyllithium was prepared following the procedure described by Winstein.[7] The 4-camphyllithium reagent in cyclohexane was filtered through the sintered glass funnel directly into a solution of cyclohexane containing 10 g. (7.2 g., 0.067 mole required) of phenyl disulfide. As the lithium reagent was added, the reaction mixture turned milky white, but very little heat was evolved. After the addition was complete, the mixture was heated under reflux for 2 to 3 hours. The reaction mixture was then cooled and added slowly to a solution of 5 g. of lithium aluminum hydride in ethyl ether to reduce the excess phenyl disulfide to thiophenol. After the reduction was complete, the excess hydride was destroyed with ethyl acetate, and the solution was poured slowly into excess 6 *N* sulfuric acid solution containing much chopped ice. After separation of the organic layer, the water layer was extracted twice with cyclohexane. The combined organic layers were then extracted five times with equal volumes of 5% sodium hydroxide solution, washed with water, and dried over anhydrous potassium carbonate. The solvent was removed at atmospheric pressure through a column, and the residue was heated gently in a subliming tube under reduced pressure (15 mm.). Unreacted 4-chlorocamphane, 2.75 g. (24% recovery), sublimed onto the walls of the tube and was carefully removed. The yellow residual oil was then distilled under reduced pressure. On redistillation, 10.2 g. (62%) of 4-phenylmercaptocamphane was obtained as a colorless oil, which could not be induced to crystallize, b.p. 119–121° (0.1 mm.), $n^{25}D$ 1.5557.

Anal. Calcd. for $C_{16}H_{22}S$: C, 78.00; H, 9.00. Found: C, 78.36; H, 8.90.

4-Benzenesulfonylcamphane (XII).—4-Phenylmercaptocamphane (1.7 g., 0.0069 mole) was treated with excess perbenzoic acid in the usual way; the reaction mixture was allowed to stand overnight at room temperature. After extraction of the benzoic acid and evaporation of the solvent under a current of air, the crude solid was recrystallized from benzene–petroleum ether, yielding 1.0 g. (52%) of long, white needles, m.p. 144–144.5°, which did not sublime.

Anal. Calcd. for $C_{16}H_{22}O_2S$: C, 69.05; H, 7.97. Found: C, 69.39; H, 7.80.

Desulfurization of 4-Phenylmercaptocamphane.—To a solution of 1.5 g. (0.0061 mole) of 4-phenylmercaptocamphane in 50 ml. of absolute ethanol was added 12 g. of Raney nickel. The mixture was refluxed with stirring for 20 hours, after which the solution was filtered (Filter-Cel), and the nickel cake rinsed twice with boiling absolute ethanol. The solution was transferred to a 500-ml. erlenmeyer flask (more than twice the required volume to contain the solution), 150 ml. of water was added, and the flask stoppered tightly and placed in the refrigerator. The small clumps of crystals which sublimed spontaneously onto the neck of the flask were scraped out on several occasions and resublimed. This procedure provided 0.45 g. (53%) of camphane, m.p. 153–154° (sealed tube), compared by mixed melting point with authentic camphane, 152–154° (sealed tube).

Desulfurization of 4-Benzenesulfonylcamphane.—To a solution of 0.4 g. (0.00143 mole) of 4-benzenesulfonylcamphane in 40 ml. of absolute ethanol was added 10 g. of Raney nickel. The mixture was refluxed with stirring for 17 hours. The reaction mixture was worked up in a manner identical with that used for the preceding desulfurization. Frequent removal of the crystals which sublimed onto the neck of the flask resulted, on resublimation, in collection of 0.12 g. (61%) of a white solid, m.p. 155° (sealed tube), which melted at 153–155° (sealed tube) when mixed with an equal portion of authentic camphane. The infrared spectra of this desulfurization product and authentic camphane, each dissolved in chloroform, were identical in every respect.

MADISON 6, WISC.

[CONTRIBUTION FROM THE SUBDEPARTMENT OF SYNTHETIC CHEMISTRY IN RELATION TO MEDICAL RESEARCH, BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO]

Synthesis of L-α-Glycerylphosphoryl-L-serine[1]

BY ERICH BAER, DMYTRO BUCHNEA AND HARVEY C. STANCER

RECEIVED OCTOBER 23, 1958

L-α-Glycerylphosphoryl-L-serine is obtained by phosphorylation of D-acetone glycerol with phenylphosphoryl dichloride and quinoline, esterification of the resulting acetone L-α-glycerylphenylphosphoryl chloride with N-carbobenzoxy-L-serine benzyl ester in the presence of pyridine, and removal of the protective groups by two catalytic hydrogenolyses, and acid hydrolysis. The infrared spectra of L-α-glycerylphosphoryl-L-serine, L-α-glycerylphosphorylethanolamine and L-α-glycerylphosphorylcholine, the structural bases of the majority of the glycerolphosphatides, are reported.

Glycerylphosphorylcholine (GPC), glycerylphosphorylethanolamine (GPE) and glycerylphosphorylserine (GPS) occur widely in bound form in nature as moieties of lecithins, cephalins, plasmalogens and phosphatidylserines. GPC[2–8,11] and GPE[3,5,9–11] have been found to occur in biological materials in free form. Evidence for the occurrence of free GPS may be forthcoming soon. Whether the choline, ethanolamine and serine esters of L-α-glycerolyphosphoric acid are intermediates in the biological synthesis or in the degradation of glycerolphosphatides, or in both, has not yet been established with certainty. Evidence obtained by Dawson[11] appears to indicate that

(1) The synthesis of L-α-glycerylphosphoryl-L-serine was described in a thesis submitted in 1955 by Harvey C. Stancer to the Department of Pathological Chemistry of the University of Toronto, Canada, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The present report incorporates improvements in the procedure introduced by Dr. Dmytro Buchnea.

(2) M. Diament, E. Kahane and J. Levy, *Compt. rend.*, **235**, 1058 (1952).

(3) P. N. Campbell and T. S. Work, *Biochem. J.*, **50**, 449 (1952).

(4) G. Schmidt, L. Hecht, P. Fallot, L. Greenbaum and S. J. Thannhauser, *J. Biol. Chem.*, **197**, 601 (1952).

(5) G. Schmidt, L. Greenbaum, P. Fallot, A. C. Walker and S. J. Thannhauser, *ibid.*, **212**, 887 (1955).

(6) E. J. King and M. Aloisi, *Biochem. J.*, **39**, 470 (1945).

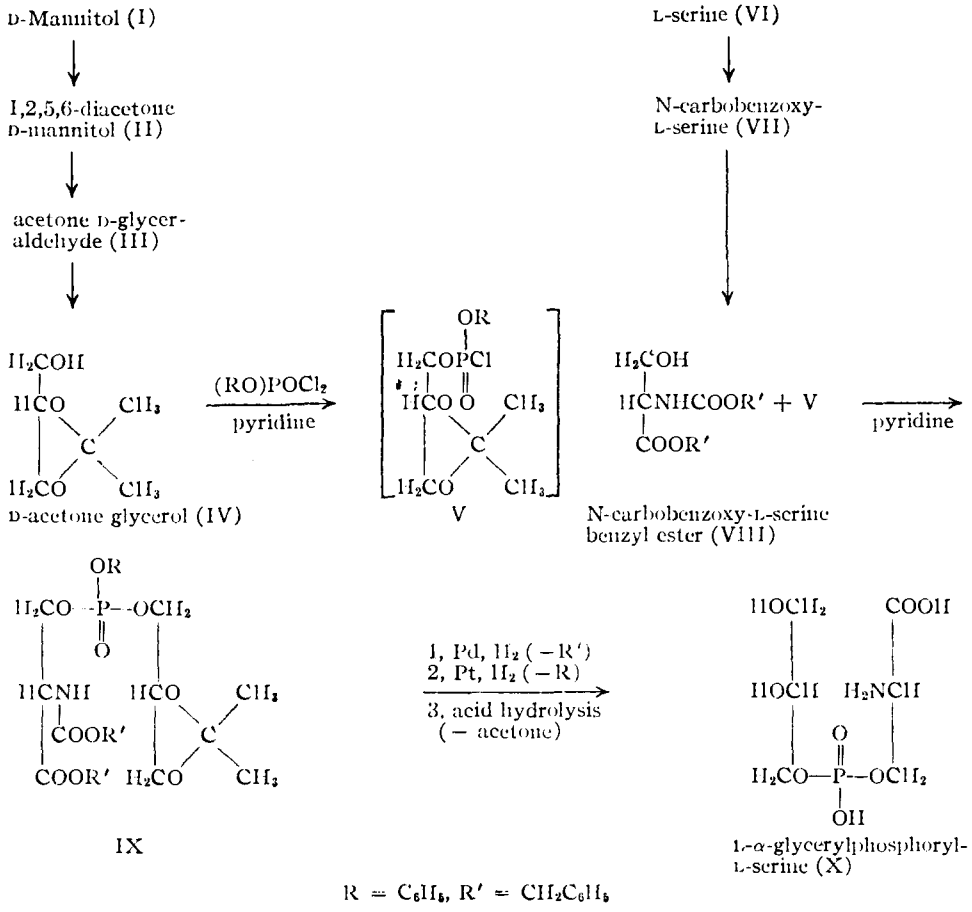
(7) G. L. Cantoni and A. W. Bernheimer, *Fed. Proc. Am. Soc. Exp. Biol. (Part II)*, **6**, No. 1, 315 (1947).

(8) R. M. C. Dawson, T. Mann and I. G. White, *Biochem. J.*, **65**, 627 (1957).

(9) D. M. Walker, *ibid.*, **52**, 679 (1952).

(10) G. B. Ansell and J. M. Norman, *ibid.*, **55**, 768 (1953).

(11) R. M. C. Dawson, *ibid.*, **59**, 5 (1955).



GPC and GPE are products of hydrolysis of phosphatides rather than intermediates in their biosynthesis.

A series of investigations in this Laboratory involving the syntheses of GPC,[12] GPE,[13] saturated and unsaturated lecithins[14,15] and cephalins,[16,17] and of phosphatidylserine,[18] and comparison of the synthetic products with the corresponding natural products established that the naturally occurring glycerolphosphatides and their basic structural units, *i.e.*, GPC, GPE and GPS, possess the α-structure and L-configuration. For the preparation of GPC and GPE, both chemical and biochemical methods are available; GPS has been observed in aqueous solution as a hydrolysis product of acetalphospholipids of brain tissue[19] and phosphatidylserine of ox-brain[20] but has not been isolated in substance. To the authors' knowledge no method for its chemical synthesis has been described.

The purpose of this paper is to report the synthesis of L-α-glycerylphosphoryl-L-serine (L-α-GP-L-S), the basic structural unit of phosphatidyl serine of ox brain.[18,21] The L-α-GP-L-S, one of four theoretically possible stereoisomers of α-GPS, was obtained by a procedure similar to those described by us for the synthesis of L-α-GPC[12] and L-α-GPE,[13] except that the choline or N-carbobenzoxyethanolamine in these procedures was replaced by the benzyl ester of N-carbobenzoxy-L-serine. The accompanying reaction scheme summarizes the synthesis of L-α-glycerylphosphoryl-L-serine from D-mannitol and L-serine, showing the last steps in detail. D-Acetone glycerol[22-24] (IV) is phosphorylated with phenylphosphoryl dichloride and quinoline, and the resulting acetone L-α-glycerylphenylphosphoryl chloride (V), without isolation, is esterified with N-carbobenzoxy-L-serine benzyl ester (VIII) in the presence of pyridine. The acetone L-α-glycerylphenylphosphoryl-N-carbobenzoxy-L-serine benzyl ester (IX) is freed of its protective benzyl and phenyl groups by two consecutive catalytic hydrogenolyses, using first palladium, then platinum as catalyst. The acetone group is removed by the acidic conditions during the hydro-

(12) E. Baer and M. Kates, THIS JOURNAL, **70**, 1394 (1948).
(13) E. Baer and H. C. Stancer, *ibid.*, **75**, 4510 (1953).
(14) E. Baer and M. Kates, *ibid.*, **72**, 942 (1950).
(15) E. Baer, D. Buchnea and A. G. Newcombe, *ibid.*, **78**, 232 (1956).
(16) E. Baer, J. Maurukas and M. Russell, *ibid.*, **74**, 152 (1952).
(17) E. Baer and D. Buchnea, *ibid.*, **81**, 1758 (1959).
(18) E. Baer and J. Maurukas, *J. Biol. Chem.*, **212**, 25 (1955).
(19) G. B. Ansell and J. M. Norman, *Biochem. J.*, **59**, No. 2, Proc. of the Biochem. Soc., 335th Meeting, page ix (1955).
(20) R. M. C. Dawson, *Biochim. et Biophys. Acta*, **14**, 374 (1954).
(21) J. Folch, *J. Biol. Chem.*, **139**, 973 (1941); **146**, 35 (1942); **174**, 439 (1948); **177**, 497 (1949).
(22) The phosphorylation of D-acetone glycerol introduces the phosphoric acid group at the hydroxyl formed by the reduction of the carbonyl group of acetone D-glyceraldehyde. It therefore is in the opposite position to the phosphoric acid group of D-glyceraldehyde-3-phosphoric acid or its reduction product D-α-glycerolphosphoric acid. Hence the phosphorylation products of D-acetone glycerol possess the L-configuration.
(23) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **128**, 475 (1939).
(24) E. Baer and H. O. L. Fischer, *ibid.*, **128**, 491 (1939).

TABLE I

L-α-GLYCEROLPHOSPHATIDES AND RELATED PHOSPHATE ESTERS NOW OBTAINABLE BY SYNTHESIS

L-α-Glyceryl-phosphoric acid[23]	Bis-(L-α-glyceryl)-phosphoric acid[31]	L-α-Glyceryl-phosphorylcholine[12]	L-α-Glyceryl-phosphorylethanol-amine[13]	L-α-Glyceryl-phosphoryl-L-serine
L-α-Phosphatidic acids	satd.[32] unsatd.[33]	L-α-Phosphatidyl-L-α-glycerols	satd.[34] unsatd.[34]	
L-α-Bisphosphatidic acids	satd.[35] unsatd.[33]	L-α-Lecithins satd.[36] unsatd.[15]	L-α-Cephalins satd.[16] unsatd.[17]	L-α-Phosphatidyl-L-serine satd.[18]

Fatty acid substituents: satd.: caproic, caprylic, capric, myristic, palmitic and stearic acid
unsatd.: oleic acid

genolyses and by final treatment of the cleavage product with Amberlite IR-120 (H^+). The chromatographically homogeneous L-α-glycerylphosphoryl-L-serine, a white and slightly hygroscopic solid substance, was obtained from D-acetone glycerol in an over-all yield of 75.5%. Its analytical values for carbon, hydrogen, nitrogen and phosphorus agreed well with those required by theory for a *monohydrate* of GPS. In aqueous solution, adjusted with sodium carbonate to a *p*H of 4.2, 1.00 mole of L-α-GP-L-S consumed in 18 hours 2.03 moles of periodic acid. One mole of periodic acid obviously is accounted for by the *vic*-hydroxyl grouping of the α-glycerol moiety. The consumption of the other mole may possibly be accounted for by either the liberated glycolaldehyde or serine after decomposition of the primary oxidation product. Oxidation of the α-amino acid grouping by periodic acid is known to occur only very slowly at room temperature. In 1 *N* hydrochloric acid at 100° the hydrolysis of L-α-GPS is complete in 1 hour.

The other three isomers of α-GPS (D-α-GP-D-S, L-α-GP-D-S, and D-α-GP-L-S) should be obtainable by the same procedure and the use of the appropriate optical isomers of acetone glycerol [L[25]; D; L] and N-carbobenzoxyserine benzyl ester [D[26]; D; L]. We have in fact carried out the preparation of a mixture of L-α-GP-L-S and L-α-GP-D-S by using DL-serine.

GPS, GPE and GPC can be separated readily from each other by paper chromatography on Whatman No. 1 paper using a solvent mixture of 99% ethanol, water and concd. ammonia (80 vol./15 vol./5 vol.), their R_f values being 0.21, 0.41 and 0.37, respectively.

To facilitate the identification and structural analysis of naturally occurring phosphatides, the infrared spectra of pure synthetic α-lecithins[15,27,28] and α-cephalins[29] have been reported by us as these substances became available. Since practically all of the known naturally occurring glycerolphosphatides are fatty acid esters of either L-α-GPC, L-GPE or L-α-GP-L-S, the infrared spectra of these compounds should be of interest as they may disclose details masked in the infrared spectra of their fatty acid esters. They are shown in Fig. 1. The infrared spectrum of L-α-GPE has been reported previously,[30] but, to allow a better comparison of the spectra, all three were recorded on the same instrument and under identical conditions. The spectra of GPS and GPC show sufficient characteristic absorptions to allow their identification and semi-quantitative determination in mixtures of all three esters, as may be obtained from the hydrolysis of lipids.

The last of the three glycerolphosphate esters that are the fundamental structural units of the known nitrogenous glycerolphosphatides has thus been synthesized. This completes a series of optically and structurally pure phosphate esters of biological interest (see Table I) that are now available by synthesis for chemical and biological studies.

Experimental

N-Carbobenzoxy-L-serine Benzyl Ester[37] (VIII).—N-Carbobenzoxy-L-serine[18] (7.17 g., 30 mmoles) was treated with 6.30 ml. of anhydrous triethylamine (45 mmoles) yielding a gum with a clear supernatant phase. Benzyl chloride (10.3 ml., 90 mmoles) was added, and the mixture was kept with occasional stirring at 75°. The initially clear and mobile reaction mixture rapidly thickened and deposited crystals. After 1.5 hours the reaction mixture was placed in a high vacuum for one hour at 70–75°, to remove the excess of reagents. The residue was partitioned between 150 ml. of ether and 75 ml. of 2 *N* hydrochloric acid, and the ethereal solution was washed successively with two 75-ml. portions each of 2 *N* hydrochloric acid, water, saturated sodium bicarbonate solution and finally with water again. The ethereal solution was dried with anhydrous sodium sulfate, and evaporated under reduced pressure to a crystalline residue, which was recrystallized from 20 ml. of hot carbon tetrachloride by the addition of 8 ml. of low-boiling petroleum ether. The N-carbobenzoxy-L-serine benzyl ester after drying weighed 8.25 g. (84%), m.p. 83.5–84.5°, $[\alpha]^{24}D$ +6.1° in dry chloroform (*c* 7); reported[18] $[\alpha]D$ +5.7° in chloroform (*c* 4). For analysis the material was recrystallized once more from carbon tetrachloride and petroleum ether.

Anal. Calcd. for $C_{18}H_{19}O_5N$ (329.3): N, 4.25. Found: N, 4.23.

Acetone L-α-Glycerylphenylphosphoryl-N-carbobenzoxy-L-serine Benzyl Ester (IX).—In a dry 200-ml. three-necked round flask equipped with an oil-sealed stirrer, calcium chloride tube and dropping funnel, was placed 9.6 g. (45.5 mmoles)

(25) E. Baer and H. O. L. Fischer, THIS JOURNAL, **61**, 761 (1939).

(26) To be prepared from N-carbobenzoxy-D-serine[18] following the procedure for the preparation of the benzyl ester of N-carbobenzoxy-L-serine described in the Experimental section of this paper.

(27) E. Baer, THIS JOURNAL, **75**, 621 (1953).

(28) E. Baer *ibid.*, **75**, 5533 (1953).

(29) E. Baer, *Can. J. Biochem. Physiol.*, **35**, 239 (1957).

(30) E. Baer and H. C. Stancer, *Can. J. of Chemistry*, **34**, 436 (1956).

(31) E. Baer and D. Buchnea, *Can. J. Biochem. Physiol.*, **36**, 243 (1958).

(32) E. Baer, *J. Biol. Chem.*, **189**, 235 (1951).

(33) E. Baer and D. Buchnea, *Arch. Biochim. et Biophys.*, **78**, 294 (1958).

(34) E. Baer and D. Buchnea, *J. Biol. Chem.*, **232**, 895 (1958).

(35) E. Baer, *ibid.*, **198**, 853 (1952).

(36) E. Baer and V. Mahadevan, THIS JOURNAL, in press.

(37) The procedure for the synthesis of N-carbobenzoxy-L-serine benzyl ester of Baer and Maurukas[18] has now been shortened considerably by following the procedure of Schwyzer, *et al.* (R. Schwyzer, M. Feurer, B. Iselin and H. Kägi, *Helv. Chim. Acta*, **38**, 80 (1955)) for the preparation of cyanomethyl esters of amino acids, and condensing N-carbobenzoxy-L-serine with benzyl chloride in the presence of an excess of triethylamine. The modified procedure for the synthesis of N-carbobenzoxy-L-serine benzyl ester was developed by Dr. R. J. Stedman.

of freshly fractionated monophenylphosphoryl dichloride[13,38,39] and 20 ml. of glass beads (6–7 mm. in diameter). The flask was cooled to −16° and a mixture of 6.08 g. (46.0 mmoles) of freshly prepared D-acetone glycerol[40] and 6.6 g. (51 mmoles) of anhydrous quinoline[41] was added dropwise with stirring to the phosphorylating agent over a period of 20 minutes, followed a few minutes later by 20 ml. of anhydrous pyridine.[42] The mixture was kept for 30 minutes at −16°, then the bath was removed and the mixture was held for 30 minutes at room temperature (25°). At the end of this period it was immersed in a bath at 15°, and a solution of 15.2 g. of N-carbobenzoxy-L-serine benzyl ester (46.1 mmoles) in 40 ml. of anhydrous pyridine was added. The funnel was rinsed with 10 ml. of pyridine. The mixture was kept for 2 hours at 15° and 1 hour at 25°. It was then diluted with 300 ml. of anhydrous ether, and the precipitate and glass beads were removed by filtration with suction. The precipitate and beads were washed with 150 ml. of ether, and the combined filtrates were concentrated under reduced pressure to a thick oil, and most of the pyridine was removed by vacuum distillation at a bath temperature of 35–40°, while the pressure was reduced gradually to 0.1 mm. The residual oil was redissolved in 500 ml. of ether, and the solution was washed in succession, as rapidly as possible, with three 300-ml. portions of ice-cold 2 *N* sulfuric acid, 300 ml. of water, two 300-ml. portions of a saturated sodium bicarbonate solution and finally with two 300-ml. portions of water. The ether solution was dried with 200 g. of anhydrous sodium sulfate, and concentrated under reduced pressure to a thick oil. The last traces of solvent were removed by keeping the oil in a vacuum of 0.2 mm. (or less) at a temperature of 35–40° until its weight was constant. The acetone L-α-glycerylphenylphosphoryl-N-carbobenzoxy-L-serine benzyl ester (IX) weighed 25.2 g. (91.5% of the theoretical yield calculated for acetone glycerol). It was found to be readily soluble in ether or chloroform, but insoluble in water; $n^{26}D$ 1.5365, $[\alpha]^{26}D$ −4.8° in 99% ethanol (*c* 10), $[\alpha]^{26}D$ +2.6° in benzene (*c* 10), $[\alpha]^{26}D$ +11.6° in anhydrous, ethanol-free chloroform (*c* 10). *Anal.* Calcd. for $C_{30}H_{34}O_{10}NP$ (599.6): C, 60.10; H, 5.72; N, 2.34; P, 5.17; acetone, 9.70. Found: C, 60.51; H, 5.97; N, 2.32; P, 5.11; acetone, 9.80.

L-α-**Glycerylphosphoryl-L-serine (X).** (1) **Removal of Both Benzyl Groups.**—A solution of 14.4 g. of acetone L-α-glycerylphosphoryl-N-carbobenzoxy-L-serine benzyl ester and of 5 ml. of glacial acetic acid in 100 ml. of 99% ethanol was placed in an all-glass reduction vessel, and 3.0 g. of freshly prepared palladium black[43] was added. The mixture was shaken vigorously in an atmosphere of pure hydrogen at a pressure of 40–50 cm. of water until the absorption of hydrogen ceased (approx. 75 min., consumed hydrogen 1100 ml., uncorrected). The hydrogen was replaced by nitrogen, the mixture was centrifuged, and the catalyst was extracted with three 45-ml. portions of dist. water, and one 25-ml. portion of 99% ethanol.

(2) **Removal of the Phenyl Group.**—The combined mother liquor and extracts, together with 3.0 g. of platinum oxide[44] were placed in a reduction vessel, and the reductive cleavage was carried out as described above. The reaction was complete at the end of 2 hours with the consumption of 4400 ml. of hydrogen (uncorrected). The hydrogen was replaced by nitrogen, the mixture was separated by centrifugation, and the catalyst was washed with three 25-ml. portions of water. The combined solutions were brought to dryness by removing the solvents under reduced pressure at a bath temperature of 35–40°. Paper chromatography of the remaining material, with a mixture of ethanol, water and concd. ammonium hydroxide (80:15:5, v./v./v.) as developing solvent, gave three ninhydrin-positive spots. For purification, the crude material was dissolved in 250 ml. of dist. water, and the solution was passed through a column (80 cm. in length, 3 cm. in width) of Amberlite (IR 120, H-form). The column was washed with 500 ml. (not more) of dist. water, the combined effluents were concentrated under reduced pressure at a bath temperature of 35–40°, and the remaining viscous material was dried *in vacuo* (0.01 mm.). The sponge-like solid was triturated with 20 ml. of 99% ethanol, the mixture was separated by centrifugation, and the precipitate was dried *in vacuo* (0.01 mm.) at room temperature over phosphorus pentoxide. The L-α-glycerylphosphoryl-L-serine (X), a hygroscopic and highly water-soluble white solid material, weighed 5.0 g. (82.5%) and was chromatographically homogeneous; over-all yield calculated from D-acetone glycerol 75.5%, $[\alpha]^{24}D$ +4.5° in *N* hydrochloric acid (*c* 10), $[\alpha]^{24}D$ −2.0° in water (*c* 10). At room temperature, it is highly soluble in water, slightly soluble in methanol and insoluble in ethanol, chloroform, acetone, ether or petroleum ether. *Anal.* Calcd. for $C_6H_{14}O_8NP \cdot H_2O$ (277.2): C, 26.00; H, 5.82; N, 5.05; P, 11.18. Found[45]: C, 26.19; H, 6.17; N, 5.01, 5.03; P, 11.12, 11.13.

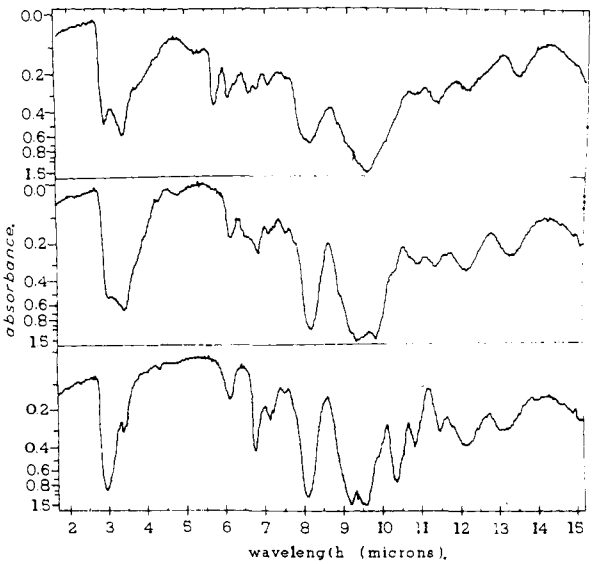


Fig. 1.—Infrared spectra of L-α-glycerylphosphoryl-L-serine (top), L-α-glycerylphosphorylethanolamine (middle) and L-α-glycerylphosphorylcholine (bottom). The instrument used was a Perkin-Elmer double beam spectrophotometer, model 21. Samples of the three esters, weighing 100 micrograms each, were dissolved in water (1 ml.), a 0.7-ml. aliquot was added to potassium bromide (25–29 mg.), the solution was lyophilized *in vacuo*, and about one-third of the material was used to prepare a disk by compressing it in a micro die for 3 minutes under vacuum at a pressure of 40 atm.

Acknowledgment.—This work was supported by a grant from the National Research Council (Ottawa), Division of Medical Research, which is gratefully acknowledged. The infrared spectra were obtained through the courtesy of Dr. G. V. Marinetti of the Department of Biochemistry, and Dr. W. B. Mason and Mr. A. Behringer of the Atomic Energy Project of the University of Rochester School of Medicine and Dentistry, and were made possible in part by funds from the United States Atomic Energy Commission.

TORONTO 5, CANADA

(38) P. Brigl and H. Müller, *Ber.*, **72**, 2121 (1939).

(39) H. Zenftman and R. McGillivray, *C. A.*, **45**, 9081 (1951); British Patent 651,656.

(40) E. Baer in "Biochemical Preparations," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1952, p. 31; see also Footnote 28 of ref. 13.

(41) Synthetic quinoline and barium oxide were shaken for 16 hours, and the quinoline was distilled *in vacuo*.

(42) Pyridine of a good commercial grade was dried by refluxing over barium oxide, and distilled with the exclusion of moisture.

(43) J. Tausz and N. von Putnoky, *Ber.*, **52**, 1573 (1919).

(44) The catalyst was prepared as described in "Organic Syntheses," Coll. Vol. I, 2nd edition, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 463, except that the sodium nitrate was replaced by an equimolecular amount of potassium nitrate; A. H. Cook and R. P. Linstead, *J. Chem. Soc.*, 952 (1934).

(45) All analytical weighings were carried out under anhydrous conditions.