1-Thiosorbitol has been found to undergo normal mercaptan reactions such as oxidation to the disulfide, acylation, and etherification with alkyl halides. In addition, it has been observed to form water-soluble salts with a variety of heavy metals.

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[CONTRIBUTION FROM THE BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO]

L-α-Glycerylphosphorylcholine

BY ERICH BAER AND MORRIS KATES[1]

Studies with labelled choline (N^{15}) and radioactive phosphorus (P^{32}) have shown that a rapid metabolic turnover of phospholipids, particularly of the small intestine, liver and kidney, takes place. These observations evoke considerable interest in the role of glycerylphosphorylcholine (G.P.C.) as an intermediary metabolite, since it is highly probable that this diester plays an essential part in the biosynthesis and the turnover of lecithins. Until quite recently (1945) an investigation of the metabolic fate of the diester was difficult because it was not obtainable in sufficient quantity or purity.

Attempts to isolate G.P.C. from biological material have been made frequently. In 1935 Contardi and Ercoli[2] incubated lysolecithin with purified rice bran extracts and observed the formation of a water-soluble organic phosphate. Although this substance was not isolated in pure state, its behavior indicated that it was a glycerylphosphorylcholine. Kahane and Lévy[3] on hydrolysis of egg yolk lecithin with lecithinase B (rat intestine) obtained a choline derivative of glycerophosphoric acid which was soluble in water, methanol, ethanol and insoluble in acetone. Further experimental work strongly suggesting the presence of G.P.C. in commercial preparations of dried beef pancreas,[4] and in tissue of fresh heart muscle of frogs and rabbits[5] has been reported.

Schmidt, Hershman and Thannhauser[6] succeeded in isolating from beef pancreas autolysates *levo*-rotatory G.P.C. in fairly pure form and were able to establish its constitution as that of the choline ester of α-glycerophosphoric acid. Utilization of a biological source, however, does not lend itself readily to the preparation of G.P.C. on a laboratory scale in amounts exceeding a few grams.

During the past ten years much of the work in this Laboratory has been directed toward the synthesis of optically pure enantiomers of asymmetrically substituted glycerol derivatives. In the desire to extend our synthetic endeavours to the field of the phospholipids and in the hope of being able to supply the biochemist with a much needed material, the synthesis of L-α-G.P.C., a substance closely related to the lecithins, was attempted.

In a previous communication[7] it was shown that the optically active α-glycerophosphoric acid isolated from lecithins belongs to the L-series and can be synthesized by phosphorylation of D(+)-acetone glycerol. The use of the latter substance insured simultaneously the position of attachment of the phosphate group and the desired L-configuration of the α-glycerophosphoric acid.[8] It was to be expected that the α-G.P.C. obtained from lecithin would have the same configuration and should be obtainable in a similar manner by esterification of phosphoric acid with D(+)acetone glycerol and choline. The synthesis, especially the phosphorylation step offered, however, a number of technical difficulties which had to be overcome before a procedure could be found which would give consistently satisfactory yields of glycerylphosphorylcholine. The method of synthesis of L-α-G.P.C. which was finally adopted and the steric relationships of the various intermediate compounds are illustrated in the accompanying reaction scheme. After trying numerous phosphorylation procedures it was found that the intermediary acetone glycerylphenylphosphorylcholine chloride (C-Cl) is obtainable in adequate amounts by phosphorylation of D(+)acetone glycerol with phenylphosphoryl dichloride in the presence of quinoline, followed by esterification of the reaction product with choline in the presence of pyridine. The isolation of the choline ester from the reaction mixture was greatly facilitated by the observation that its reineckate, in contrast to the reineckates of pyridine and quinoline, precipitates from an alkaline–aqueous solution and can be separated from the similarly alkali-insoluble reineckates of choline and other choline-containing reaction products by means of its solubility in ethyl acetate. The reineckate of (C) was converted to the corresponding sulfate (C-SO_4/2) before removing the protective phenyl and acetone groups in order to avoid complications introduced

(1) This paper forms part of a thesis which will be submitted by M. Kates to the Department of Chemistry, University of Toronto, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. An account of this work was presented before the Canadian Physiological Society, at the London (Ontario) meeting, October 24–25, 1947.

(2) A. Contardi and A. Ercoli, *Arch. sci. biol.*, **21**, 1 (1935).

(3) E. Kahane and J. Lévy, *Compt. rend.*, **219**, 431 (1944).

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

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

(6) G. Schmidt, B. Hershman and S. J. Thannhauser, *J. Biol. Chem.*, **161**, 523 (1945).

(7) E. Baer and H. O. L. Fischer, *ibid.*, **128**, 491 (1939).

(8) An optically active α-monoglyceride is considered as being related to the glyceraldehyde which would be obtained by oxidation of the γ-carbon atom.

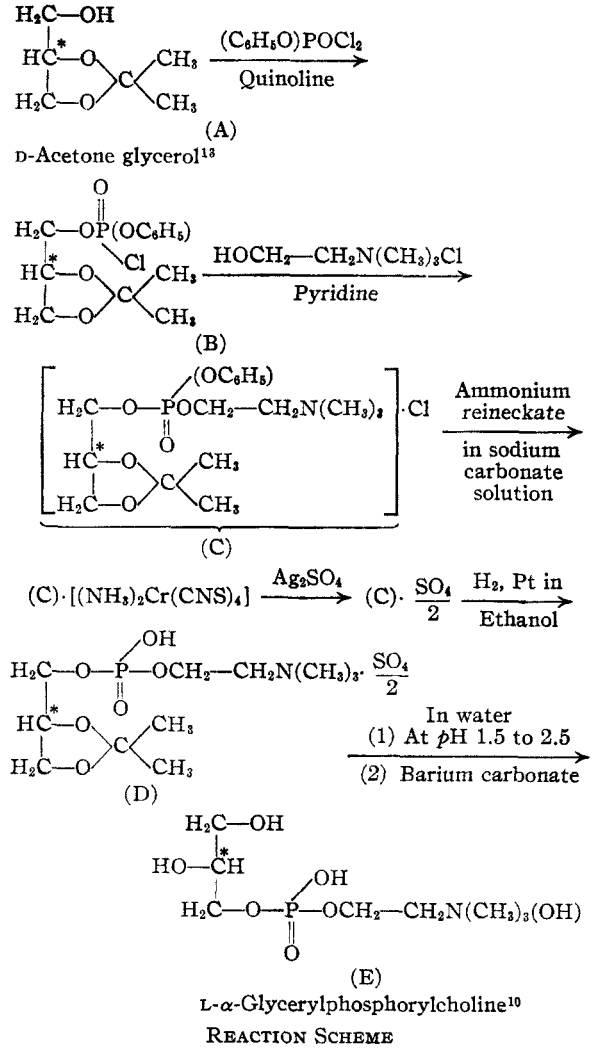
by the reineckate ion. It was found that the order of removal of these groups was a decisive factor in obtaining L-α-glycerylphosphorylcholine. All attempts to prepare the diester by removing first the acetone group of (C-SO_4/2) failed because, at the *p*H required for its removal, liberation of choline and $\alpha \leftrightarrows \beta$ migration of phosphoric acid took place. In contrast, when the phenyl group of (C-SO_4/2) was removed first, the resulting acetone glycerylphosphorylcholine (D-SO_4/2) was found to be stable enough to permit its deacetonation within the *p*H-range of 1.5–2.5 without simultaneous liberation of choline or phosphoric acid migration to give a good yield of the diester (E).

The synthetic L-α-G.P.C. was obtained as a colorless, hygroscopic and viscous liquid in an over-all yield varying from 35–40%; $[\alpha]^{23}D$ −2.85° (±0.1°) in water (average of 15 preparations). The diester is fairly stable in aqueous solution at room temperature within the *p*H-range of 1.5 to 7; in alkaline solution or in strongly acid solution, however, it is rapidly hydrolyzed. The cleavage of its choline-phosphoric acid linkage is also effected by the recently described enzyme preparation from carrots.[9] The diester is precipitated from alcoholic solution by ammonium reineckate or cadmium chloride.

The synthetic L-α-G.P.C. was shown to contain neither inorganic phosphate nor free choline. It analyzed correctly for $C_8H_{22}O_7NP$ and its molecular ratio of *choline : phosphoric acid : α-glycerol ester* corresponded very closely to the theoretical value of 1:1:1. The diester was further characterized by means of an amorphous cadmium chloride compound ($[\alpha]D$ −1.2°) and a crystalline cadmium chloride compound (m. p. 100–102°; $[\alpha]D$ −1.4°), both of which were obtained in excellent yields. On the basis of the analytical data formula $[C_8H_{22}O_7NP]_2 \cdot [CdCl_2]_3$ had to be assigned to the amorphous compound and formula $[C_8H_{22}O_7NP][CdCl_2] \cdot 2H_2O$ to the crystalline compound. On decomposition of the two cadmium chloride addition compounds with silver carbonate the L-α-G.P.C. was recovered unchanged ($[\alpha]D$ −2.9°).

The properties of the synthetic L-α-G.P.C. were similar to those described by Schmidt, Hershman and Thannhauser for the natural G.P.C. except that the rotation of the synthetic diester was considerably lower than that reported for the biological diester ($[\alpha]D$ − 4.87°). This discrepancy could be accounted for either by a partial inactivation of the synthetic α-diester during the later stages of the synthesis or by contamination of the biological diester with compounds of high optical activity.

First of all attempts were made to establish the optical purity of the synthetic diester by degradation to the well known L-α-glycerophosphoric acid.[7] The glycerophosphoric acid obtained by



REACTION SCHEME

acid or alkaline hydrolysis had, however, a much lower rotation than that reported for the synthetic compound and, depending on the method of hydrolysis, containing varying proportions of L-α-, D,L-α- and β-glycerophosphoric acid. The formation of β-glycerophosphoric acid from pure α-G.P.C. must have been caused by acyl-migration during hydrolysis. In order to prevent this migration attempts were made to block both alcoholic hydroxy groups by methylation. The low solubility of the G.P.C. in all solvents commonly used in etherification procedures made the complete methylation of the glycerol-moiety impossible. After several other unsuccessful attempts to establish the optical purity of the synthetic L-α-G.P.C. by relating it to L-α-glycerophosphoric acid, work in this direction was abandoned.

It was then decided to repeat the isolation of G.P.C. from autolyzed beef pancreas as described

(9) D. J. Hanahan and I. L. Chaikoff, *J. Biol. Chem.*, **168**, 233 (1947); **169**, 699 (1947).

(10) The guiding principles in establishing the steric classification of the enantiomeric glycerides and related compounds are outlined by H. O. L. Fischer and E. Baer in *J. Biol. Chem.*, **128**, 475 (1939), and in *Chem. Rev.*, **29**, 287 (1941).

by Schmidt, Hershman and Thannhauser. In view of the complex nature of the autolysate, it was considered possible that small amounts of impurities of high optical activity might still be associated with the product obtainable by this procedure. The rotation of the G.P.C. obtained by us was even higher than that reported by Schmidt, *et al.* However, several repetitions of the Amberlite treatment gradually removed the basic impurities and lowered the rotation to a point where it became not only constant ($[\alpha]_D$ $-2.8°$) but was in complete agreement with that of the synthetic diester. Furthermore, the crystalline cadmium chloride addition compound obtained from the highly purified natural diester was identical with the corresponding compound of the synthetic glycerylphosphorylcholine. The identity of the natural levorotatory G.P.C. and the synthetic L-α-G.P.C. was thus established beyond doubt. The L-configuration, as anticipated, must therefore be assigned to the natural diester. The fact that the optical activities of both compounds, each obtained by a different procedure, and those of their derivatives are in complete agreement suggests with high probability that the synthetic L-α-G.P.C. is optically pure, the possibility that both compounds have been inactivated to the same extent being remote.

By means of the same series of reactions as described for the synthesis of L-α-G.P.C., but starting with L(−)acetone glycerol or racemic acetone glycerol, D-α- or D,L-α-G.P.C. are obtainable. In the course of the present investigation the racemic α-G.P.C. has been prepared. Since, however, its synthesis is identical with that of the optical isomer only the physical and analytical data of the diester and its intermediary compounds are reported.

A kinetic study of the acid and alkaline hydrolysis of the L-α-G.P.C. has shown that the liberation of choline is accompanied by a reversible phosphoric acid migration resulting in the formation of a mixture of L-α-, D,L-α- and β-glycerophosphoric acid. The close relationship of α-G.P.C. to lecithins permits the prediction of similar chemical changes on subjecting lecithins to acid or alkaline hydrolysis. A detailed account of this work which seems to invalidate the methods commonly used in the elucidation of the structure of lecithins, will be published elsewhere. In the light of these findings a critical re-examination of the data in the literature has raised serious doubts as to the natural existence of β-lecithins.

The synthesis of the enantiomeric forms (as well as the racemic form) of α-lysolecithins[11] and α-lecithins of known constitution and configuration *via* the corresponding enantiomers of α-G.P.C. has now become possible. Work along these lines is in progress in this Laboratory.[12] The synthetic α-glycerylphosphorylcholines and the synthetic α-lecithins should be ideal substrates in studies concerning the specificity of the enzymes responsible for the cleavage of the various phosphatide linkages.

Finally it should be mentioned that the synthesis described in this paper should make possible the preparation of α-G.P.C. or of α-lecithins with all or some of their groups labelled by the use of (1) acetone glycerol containing deuterium,[13] (2) choline with heavy nitrogen, (3) phenylphosphoryl dichloride with radioactive phosphorus and (4) fatty acids containing deuterium or preferably heavy carbon.

Experimental Part

I. Synthesis of L-α-Glycerylphosphorylcholine

Monophenylphosphoryl Dichloride.[14]—The chloride was prepared according to Jacobsen,[15] using, however, the slightly modified procedure reported by Brigl and Müller,[14] which yields in approximately equal amounts monophenylphosphoryl dichloride and diphenylphosphoryl monochloride. The acid chlorides were separated and carefully purified by fractional distillation *in vacuo*. Boiling point of the pure phenylphosphoryl dichloride 107–109° (9 mm.)).

D(+)Acetone Glycerol.—The glycerol derivative was prepared according to the simplified procedure reported by Fischer and Baer.[16] The reduction, however, was carried out at atmospheric pressure, using Raney nickel catalyst.[17] It should be noted that the yields of L-α-glycerylphosphorylcholine reported in this communication are obtainable only by using preparations of D(+)acetone glycerol with specific rotations ranging from +13.5° to +14.0°. Preparations of lower optical activity contain moisture; their use reduces greatly the yield of the diester.

Acetone Compound of L-α-Glycerylphenylphosphorylcholine: Phosphorylation, Step 1.—In a 500-ml. round-bottomed, two-necked and thick-walled flask equipped with a mercury-sealed, motor-driven stirrer and dropping funnel were placed 18.2 ml. (0.123 mole) of monophenylphosphoryl dichloride, 16.2 ml.[18] (0.138 mole) of dry quino-

(11) The α indicates the position of the phosphoric acid.

(12) Attempts to prepare optically active lecithins via the enantiomeric forms of α,β diglycerides by means of the double phosphorylation procedure are also being made.

(13) H. Erlenmeyer, H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, **20**, 1012 (1937).

(14) Phosphorus oxychloride, widely used as a phosphorylating agent, has the disadvantage of giving rise to the formation of phosphorus-containing by-products which are not only difficult to remove but also reduce the yield considerably. Most of the undesired effects associated with the use of this agent may be avoided by utilizing its phenyl esters. The successful use of the diphenylphosphoryl-chloride as a phosphorylating agent has been reported frequently during recent years but the first successful application of phenylphosphoryl dichloride for the preparation of *mixed* diesters of phosphoric acid will be described in this communication. *Cf.* P. Brigl and H. Müller, *Ber.*, **72**, 2121 (1939). This reagent should prove useful in the synthesis of other compounds of biological interest.

(15) G. Jacobsen, *Ber.*, **8**, 1519 (1875).

(16) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **128**, 463 (1939).

(17) A detailed description of the most recent procedure for the preparation of D(+)acetone glycerol will appear in "Biochemical Preparations" as a part of the preparation of L-α-glycerophosphoric acid.

(18) Quinoline of a good commercial grade was dried over potassium hydroxide and fractionated within narrow limits of boiling point. By substituting pyridine for quinoline in Step 1, only very small amounts of the acetone compound of glycerylphenylphosphorylcholine are formed in Step 2, presumably because of the increased formation of di-(acetone-glyceryl)-phenylphosphate in Step 1. This assumption is supported by the observation of E. Fischer and E. Pfähler, *Ber.*, **53**, 1606 (1920), that the tendency of phosphorus oxychloride to react simultaneously with more than one of its chloride groups is greater in pyridine than in quinoline.

line[18] and 50 to 60 ml. of glass beads (6–7 mm. diameter).[19] The flask was immersed in a cold bath (−10°) and 16.15 g. (0.123 mole) of freshly prepared D(+)acetone glycerol was added dropwise in the course of four to five minutes to the vigorously stirred phosphorylating mixture. After five minutes the cold-bath was removed and the mixture allowed to come to room temperature. The reaction product, acetone-L-α-glycerylphenylphosphoryl chloride, was not isolated.

Phosphorylation, Step 2.—The reaction mixture was immediately broken up as quickly as possible, covered with 100 ml. of dry pyridine[20] and vigorously stirred until a fine suspension was formed. To this suspension were added 15.8 g. (0.115 moles) of dry choline chloride[21] and 90 to 100 ml. of glass beads. The stirring was continued for a period of at least forty hours.[22]

Isolation of the Phosphorylation Product as Reinecke Salt.—The reaction flask was attached to a receiver and the mixture concentrated *in vacuo* (bath 40°) to a sirup. The residue was poured with stirring into 450 ml. of an ice-cold sodium carbonate solution (60 g. of anhydrous sodium carbonate in 600 ml. of water). The remainder of the carbonate solution was used to rinse the flask and glass beads. The combined aqueous solutions were freed from suspended quinoline by centrifugation and the aqueous layer poured through a wet filter into a freshly prepared solution of 53–55 g. of ammonium reineckate[23] in 1800 ml. of distilled water containing 10 g. of sodium carbonate.[24] After the addition of a small amount of filter-aid (Hyflo-Super-Cel) the mixture was filtered with suction. The precipitate was washed thoroughly with water and dried *in vacuo* over solid sodium hydroxide and phosphorus pentoxide to constant weight. The dry reineckate was powdered, extracted by stirring with 700 ml. of dry ethyl acetate[25] and the suspension was sharply centrifuged. The extraction of the reineckate was repeated with successively smaller amounts of ethyl acetate until the extracts were only faintly colored. Seven to eight extractions, using a total of 2200 ml. of ethyl acetate, were required. The combined extracts, if necessary, were cleared by centrifugation. The supernatant liquid was concentrated *in vacuo* to a volume of approximately 100 ml. and the concentrate diluted gradually with 500 ml. of dry and ethanol-free ether. The precipitate was filtered off with suction, washed thoroughly on the filter with ether and dried *in vacuo*. The yield of already fairly pure reinecke salt of acetone-L-α-glycerylphenylphosphorylcholine varied from 38 to 47 g. (45 to 55%); m. p. 136.5–137.5°. The reineckate is readily soluble in acetone or ethyl acetate, less soluble in ethanol and insoluble in water, ether or benzene. For analytical purposes only, the reineckate was crystallized from 95% ethanol; prisms, m. p. 137.0–137.5°.

Anal. Calcd. for $C_{21}H_{35}O_6N_7S_4PCr$ (692.6): C, 36.4; H, 5.52; N, 14.15; P, 4.47. Found: C, 36.5; H, 5.32; N, 14.08; P, 4.41.[26]

Conversion of the Reineckate to the Sulfate.—Ten grams of the reineckate[27] was dissolved in 40 ml. of acetone and the solution was diluted with 60 ml. of 95% ethanol. To this solution was added gradually and with cooling a lukewarm 1% aqueous silver sulfate solution (approximately 225 ml.) until the precipitation of the silver reineckate was complete. The precipitate was removed by centrifugation, washed with 95% ethanol and the combined supernatants were concentrated *in vacuo* as rapidly as possible to a volume of approximately 50–60 ml. at a bath temperature not exceeding 40°.[28] Remaining traces of the original reineckate were decomposed by the dropwise addition of a dilute silver sulfate solution. The silver reineckate was removed, the solution taken to dryness under reduced pressure (bath 35 to 40°) and the residue dried in a vacuum of 0.5 mm. The crude sulfate (5.1 g.) was dissolved in 25–30 ml. of 99% ethanol, freed from insoluble material (100–300 mg.) and the solution taken to dryness *in vacuo*. At this stage the sulfate (4.8 g., 79%) is a glass-like mass which is pure enough for further processing. The sulfate can be obtained in crystalline state by taking it up with warm, dry acetone (7 ml./g.) and keeping the mixture overnight in the ice-box (+5°); recovery approximately 85%.

For analytical purposes the crystalline sulfate was purified further by dissolving it in 99% ethanol, centrifuging the suspension, evaporating the supernatant liquid *in vacuo* to a small volume and adding gradually dry acetone to the concentrate until crystallization set in. After five minutes another portion of dry acetone equal to the first was added and the mixture kept in an ice-box for twenty-four hours. The hygroscopic crystals were filtered rapidly with suction, washed with a small portion of anhydrous acetone and dried *in vacuo* over fresh calcium chloride. Recovery of sulfate approximately 50%; m. p. 108–109.5° (sint. 101°); $[\alpha]^{27}D$ −8.3° in water (*c*, 7.8); $[\alpha]^{28}D$ −3.0° in dry ethanol (*c*, 6.1).[29]

The strongly hygroscopic sulfate is readily soluble in water or ethanol, sparingly soluble in cold acetone or dioxane and insoluble in ether. *Anal.* Calcd. for $C_{34}H_{68}O_{16}N_2P_2S$ (844.5): C, 48.25; H, 6.91; N, 3.31; P, 7.34; SO_4 11.37; acetone, 13.70; choline, 28.8. Found: C, 48.95; H, 7.06; N, 3.29; P, 7.17; SO_4 11.18; acetone, 13.35; choline,[30] 28.8.

L-α-Glycerylphosphorylcholine: Removal of the Phenyl Group by Reductive Cleavage.—Sixteen grams of the L-α-glycerylphenylphosphorylcholine reineckate[27] was converted into the crude sulfate as described above. The sulfate (8.5 g.) was dissolved in 80 ml. of 99% ethanol and freed by centrifugation from insoluble material. The clear solution together with 2 g. of platinic oxide (Adams catalyst) was shaken vigorously in an atmosphere of pure hydrogen at room temperature and a pressure of 40 to 50 cm. of water in excess of atmospheric

(19) By the use of glass beads the choline chloride is brought into a finely dispersed state and the formation of a sticky gum, which would enclose unreacted material, is minimized. The efficiency of the phosphorylating procedure is thus greatly increased.

(20) This base rather than quinoline was used in the second step of the phosphorylation because of the greater activity of the phosphorus oxychlorides in pyridine. Pyridine of a good commercial grade was refluxed over barium oxide and distilled with exclusion of moisture.

(21) The choline chloride was thoroughly dried *in vacuo* over phosphorus pentoxide at 56°.

(22) The reaction vessel was partially immersed in a large water-bath (20–25°) to prevent a rise in temperature due to the friction of the glass beads. Otherwise a marked darkening of the reaction mixture occurs.

(23) The commercial ammonium reineckate is often not sufficiently pure. It was found more economical to prepare the ammonium salt as described in "Organic Syntheses," Coll. Vol. II, p. 555.

(24) The alkalinity of the dilute sodium carbonate solution suffices to prevent the precipitation of pyridine reineckate and quinoline reineckate.

(25) Ethyl acetate, if moist, also dissolves some of the impurities. It suffices to dry the commercial ethyl acetate with anhydrous potassium carbonate.

(26) The P-determination on the reineckate was carried out according to King (*Biochem. J.*, **26**, 292 (1932)). The determination, however, was somewhat complicated by the presence of chromic acid anhydride, one of the digestion products. At the completion of the digestion the cooled solution was filtered through a sintered glass filter and the chromic anhydride crystals washed with small amounts of 60% perchloric acid. The filtrate and washings were made up to volume and used for the colorimetric determination of phosphorus.

(27) The reineckate obtained directly from the ethyl acetate extraction may be used here.

(28) The aqueous solution of the sulfate is acid. To avoid hydrolysis resulting in the liberation of acetone and choline all operations should be carried to completion as rapidly as possible and at the lowest possible temperature.

(29) The readings were taken immediately after preparing the solutions.

(30) The substance was hydrolyzed in 1.5 *N* hydrochloric acid at 100° (two hours) and the choline determined gravimetrically in form of its reineckate as described by Schmidt, Hershman and Thannhauser.

pressure until the absorption of hydrogen ceased. In about seventy-five minutes 2010 ml. (N. T. P.) or 93% of the theoretical amount of hydrogen were taken up. The catalyst was filtered off, washed with ethanol and the combined filtrate and washings evaporated to dryness *in vacuo* (bath 40°). The residue, a colorless glass weighing 6.0 g., contained in general 20% less acetone than calculated for the acetone compound of L-α-glycerylphosphorylcholine sulfate. No attempt was made to isolate a pure compound.

Deacetonation.—The crude acetone compound (6.0 g.) was dissolved in 150 ml. of distilled water and the solution, which had a *p*H of 1.5, was allowed to stand at room temperature (20 to 25°) for a period of fifteen hours.[31]

To remove traces of nitrogenous impurities, a dilute solution of ammonium reineckate was then added dropwise until the precipitation was complete and after centrifugation, the excess of ammonium reineckate was removed with dilute silver sulfate solution. The supernatant was triturated with barium carbonate until free from sulfate ions and the silver ions were removed with hydrogen sulfide in the presence of the barium salts. The mixture was centrifuged and the aqueous solution concentrated under reduced pressure (bath 35–40°) to a small volume. The deposit of insoluble material (mostly barium carbonate) was removed and the concentration *in vacuo* continued. The drying was completed in a vacuum of 0.1 mm. at a bath temperature not exceeding 40°; yield 4.0 to 4.6 g. of L-α-glycerylphosphorylcholine (65 to 75% of the theoretical from reineckate or 35 to 40% over-all yield). The synthetic diester is a viscous liquid which is readily soluble in water, ethanol or methanol and insoluble in acetone, ether or benzene; $[\alpha]^{23}D$ $-2.85 \pm 0.1°$ in water (*c*, 2.2 determined from P-content; *p*H 6–7). The optical activity of α-G. P. C. seems to decrease slightly with increasing acidity.

Anal. Calcd. for $C_8H_{22}O_7NP$ (275.2): choline, 44.0; P, 11.27. Found: choline, 42.4; P, 10.95.

Vicinal Glycol Titration with Periodic Acid.—0.1157 gram of the diester was dissolved in water and the volume made up to 100 ml. The titration was carried out according to Voris, Ellis and Maynard[32] on 10.0-ml. aliquots. After one hour 0.0422 mM. of the diester had consumed on the average 0.0414 mM. of periodic acid or 98.2% of the theoretical amount calculated for the α-glycerylphosphorylcholine. Ratio of choline:P:α-glycerol ester[33]: calcd. 1:1:1. Found: 0.99:1.00:1.01.

Amorphous Cadmium Chloride Addition Compound of L-α-Glycerylphosphorylcholine.—A solution of 5.8 g. of cadmium chloride (2.5-H_2O) in 4 ml. of water, diluted with 65 ml. of 99% ethanol, was added slowly and with stirring to a solution of 4.0 g. of L-α-glycerylphosphorylcholine in 75 ml. of 99% ethanol. After standing in the ice-box for one hour the dense, white precipitate was filtered with suction, washed with ethanol and ether, and dried *in vacuo;* yield of the amorphous cadmium chloride addition compound 7.3 g. (92%). This compound is quite stable and can be used advantageously for the storage of L-α-glycerylphosphorylcholine. If need arises it can be quickly converted into the free diester; $[\alpha]^{25}D$ $-1.2°$ in water (*c*, 5.0). *Anal.* Calcd. for $(C_8H_{22}O_7NP)_2 \cdot (CdCl_2)_3$ (1100): C, 17.44; H, 4.03; N, 2.54; P, 5.62; Cl, 19.35; Cd, 30.60; choline, 21.9; ratio of $CdCl_2:C_8H_{22}O_7NP = 3:2$. Found: C, 17.6; H, 4.24; N, 2.58; P, 5.58; Cl, 19.90; Cd, 30.15; choline, 21.1; ratio of $CdCl_2:C_8H_{22}O_7NP = 3.01:2.00$. Calcd. for the cadmium chloride-free moiety, $C_8H_{22}O_7NP$ (275): C, 34.9; H, 8.05; N, 5.08; P, 11.27; choline, 44.0. Found by calculation from the analytical values above: C, 35.2; H, 8.48; N, 5.17; P, 11.17; choline, 42.2.

Vicinal-Glycol Titration with Periodic Acid.—The sample of the cadmium chloride derivative in aqueous solution was freed from cadmium by the addition of potassium carbonate. The filtrate was made up to a known volume and the content of diester ascertained by a phosphorus determination. Several aliquots each containing 0.0218 mM. of the diester consumed in two hours on the average 0.0201 mM. (96.3%) of periodic acid.

Crystalline Cadmium Chloride Compound of L-α-Glycerylphosphorylcholine: (a) Prepared from the Amorphous Cadmium Chloride Compound.—A solution of 3.1 g. of the *amorphous* cadmium chloride compound in 38 ml. of water was diluted gradually with 150 ml. of 99% ethanol and a small amorphous precipitate removed immediately by centrifugation. The clear supernatant liquid was first kept at room temperature for twenty-four hours, during which time crystals (prisms) began to form and was then kept in an ice-box (+5°) for two days. The crystals were filtered with suction, washed with a small volume of cold 80% ethanol and *dried in air* to constant weight. The crystalline cadmium chloride compound of L-α-glycerylphosphorylcholine was obtained in a yield of 1.85 g. (66.4%); m. p. 100–102° with sintering from 97° (rise in temperature 3°/min. starting with a bath temperature of 80°); $[\alpha]^{24}D$ $-1.4°$ in water (*c*, 5.5). *Anal.* Calcd. for $(C_8H_{22}O_7NP)(CdCl_2) \cdot 2H_2O$ (494.7): C, 19.46; H, 5.30; N, 2.83; P, 6.27; Cl, 14.33; Cd, 22.7; choline, 24.5. Found: C, 19.31; H, 5.21; N, 2.84; P, 6.28; Cl, 14.50; Cd, 22.8; choline, 24.6. Ratio of $C_8H_{22}O_7NP:CdCl_2$. Calcd. 1.0:1.0. Found. 1.00:1.01. The air-dried cadmium chloride compound lost on drying over phosphorus pentoxide in a vacuum of 0.1 mm. at 56° 10.62% of its weight. Calcd. for a loss of three moles of water 10.92%.[34]

Vicinal-Glycol Titration with Periodic Acid.—Carried out as described for the amorphous cadmium chloride compound. At the end of two hours 0.0235 mM. of the diester had consumed on the average 0.0233 mM. (99.2%) of periodic acid.

(b) Prepared directly from the diester. To the combined solutions of 2.0 g. of the diester in 13 ml. of water and of 2.6 g. of cadmium chloride (2.5 H_2O) in 15 ml. of water were added gradually and with swirling 100 ml. of 99% ethanol. The solution was immediately cleared of a small amount of amorphous material by centrifugation. The supernatant liquid was diluted with an additional portion of 10 ml. of 99% ethanol and crystallization induced mechanically. After the mixture had stood for six hours at room temperature and twenty-four hours in the ice-box the crystals (prisms) were filtered with suction, washed with 80% ethanol and dried in air to constant weight; yield of crystalline cadmium chloride compound 80% (2.8 g.); m. p. 97–101° (sintered 94°); $[\alpha]^{27}D$ $-1.4°$ in water (*c*, 5.8). *Anal.* Found: C, 19.14; H, 5.25; N, 2.85; P, 6.22.

Recovery of L-α-Glycerylphosphorylcholine from its Cadmium Chloride Compound.—To a solution of 1.0 g. of the *amorphous* cadmium chloride compound in 35 ml. of water was added 1.6 g. of silver carbonate and the mixture was stirred vigorously until free from chloride ions. After removal of the solids the solution was freed from cations with hydrogen sulfide and the sulfides removed by filtration over Hyflo-Super-Cel. The filtrate was concentrated to a sirup under reduced pressure (bath 35–40°) and the residue was dried to constant weight in a vacuum of 0.1 mm. at a temperature not exceeding 40°. In the event that the residue was still slightly colored by colloidal material it was taken up in ethanol, centrifuged, concentrated and dried *in vacuo* as described above. The recovery of L-α-glycerylphosphorylcholine was 98% (0.49 g.); $[\alpha]D$ $-2.9°$ in water (c, 2.6).

(31) According to our experience a complete hydrolysis of acetone without liberation of choline or migration of phosphoric acid is achieved within the *p*H-range of 1.5 to 2.5 at the stated time interval and temperatures. Hydrolysis at greater acidity liberates choline and at lower acidity is incomplete with regard to acetone.

(32) L. Voris, G. Ellis and L. A. Maynard, *J. Biol. Chem.*, **133**, 491 (1940).

(33) Determined by the periodic acid titration.

(34) The loss of the third mole of water during the process of drying may be explained by the formation of an inner salt of the glycerylphosphorylcholine.

II. Synthesis of D,L-α-Glycerylphosphorylcholine

The synthesis of D,L-α-glycerylphosphorylcholine is identical with that described for the L-form, except that D,L-acetone glycerol[35] is used as starting material. Only the analytical and physical data of the various compounds will be reported here.

Acetone Compound of D,L-α-Glycerylphenylphosphorylcholine: (a) **Reineckate.**—Yield 56%; m. p. 136–137°. *Anal.* Calcd. for $C_{21}H_{35}O_6N_7PCr$ (692.6): C, 36.4; H, 5.52; N, 14.15; P, 4.47. Found: C, 36.6; H, 5.49; N, 14.13; P, 4.40.

(b) **Sulfate.**—Obtained by the decomposition of the reineckate in yields of 70–80% (oil) or 60–68% (crystals). *Anal.* Calcd. for $C_{84}H_{88}O_{16}N_2P_2S$ (844.5); P, 7.34; SO_4, 11.37; acetone, 13.70; choline, 28.8. Found: P, 7.25; SO_4, 11.00; acetone, 13.76; choline, 28.2.

D,L-α-Glycerylphosphorylcholine.—Over-all yield 35–40%; viscous liquid, soluble in water, ethanol, methanol; insoluble in acetone, ether or benzene. **Amorphous cadmium chloride compound.**—Yield 90%. *Anal.* Calcd. for $(C_8H_{22}O_7NP)_2(CdCl_2)_3$: C, 17.44; H, 4.03; N, 2.54; P, 5.62; Cl, 19.35; Cd, 30.60; choline, 21.9. Found: C, 17.68; H, 4.16; N, 2.64; P, 5.72; Cl, 19.45; Cd, 30.05; choline, 21.8. Theoretical values for the cadmium chloride-free moiety $C_8H_{22}O_7NP$: C, 34.9; H, 8.05; N, 5.27; P, 11.27; choline, 44.0. Found by calculation from the analytical values above: C, 35.0; H, 8.23; N, 5.23; P, 11.32; choline, 43.4.

Vicinal-Glycol Titration with Periodic Acid.—Carried out as described for the corresponding cadmium chloride compound of the synthetic L-α-glycerylphosphorylcholine. Aliquots containing 0.0226 mM. of D,L-α-glycerylphosphorylcholine consumed in 15, 45, 90 minutes 0.0204 mM. (90.4%), 0.0215 mM. (95.2%) and 0.0217 mM. (95.9%) of periodic acid, respectively.

III. Isolation of α-Glycerylphosphorylcholine from Beef Pancreas According to Schmidt, Hershman and Thannhauser[6]

Five pounds of beef pancreas treated as outlined by Schmidt, Hershman and Thannhauser yielded 2.9 g. of crude α-glycerylphosphorylcholine with a rotation of $[\alpha]^{25}D$ −7.8° in water (*c*, 2.7). This rotation, much higher than that reported by Schmidt, Hershman and Thannhauser ($[\alpha]^{20}D$ −4.87°), indicated that our biological product still contained extraneous material of high optical activity and needed further purification. The diester was dissolved in 40 ml. of water and a dilute aqueous ammonium reineckate solution added until no further precipitation took place. The excess of ammonium reineckate was removed with dilute silver sulfate solution; the filtrate was triturated with barium carbonate and the silver ions removed with hydrogen sulfide in the presence of the barium salts. The aerated aqueous solution of the diester (approx. volume 60 ml.) was stirred for one hour with 20 g. of Amberlite (I. R.-100), filtered and the filtrate concentrated *in vacuo* at a bath temperature not exceeding 40°. The colorless oil, which weighed 1.15 g. and now had an optical activity of $[\alpha]^{25}D$ −4.2° in water, (*c*, 2.3) was again treated in aqueous solution (60 ml.) with 20 g. of Amberlite for a period of thirty minutes. The filtrate was concentrated *in vacuo* to a volume of 5 ml. and the diester precipitated by the addition of 50 ml. of dry acetone. The precipitate, freed *in vacuo* from solvent, weighed 0.7 g. and had an optical activity of $[\alpha]^{25}D$ −2.7° in water (*c*, 2.5, *p*H 2.8). This rotation, although considerably lower than that reported by Schmidt, Hershman and Thannhauser, is, however, identical with that of our synthetic product $[\alpha]^{25}D$ −2.85° (±0.1°). To ensure that the progressive decrease in optical activity was due to the removal of impurities and not to inactivation of the glycerylphosphorylcholine by the Amberlite, the oil (0.7 g.) was treated once more with the ion-exchanger. The optical activity of the recovered diester (0.6 g.) remained unchanged, $[\alpha]^{25}D$ −2.7° in water (*c*, 2.7, *p*H 2.5) or $[\alpha]^{25}D$ −2.8° (*c*, 2.6; *p*H 5.8). The fact that the optical activity of the synthetic L-α-glycerylphosphorylcholine also remained unchanged after a treatment with Amberlite is further evidence of the harmlessness of this treatment.

Crystalline Cadmium Chloride Compound of the Natural α-Glycerylphosphorylcholine.—For the purposes of analysis and further comparison of the natural L-α-glycerylphosphorylcholine with the synthetic L-α-glycerylphosphorylcholine the natural diester (0.5 g.) was converted via the amorphous cadmium chloride compound (0.88 g.) to the crystalline cadmium chloride addition compound (prisms, 0.56 g.) as described for the synthetic product; m.p. of the crystalline cadmium chloride compound 99–100° (sintered at 90°; rise in temperature 3°/minute, starting with a bath of 80°); $[\alpha]^{24}D$ −1.4° in water (*c*, 5.5). *Anal.* Calcd. for the crystalline cadmium chloride compound $(C_8H_{22}O_7NP)(CdCl_2)\cdot 2H_2O$ (494.7): C, 19.46; H, 5.30; N, 2.83; P, 6.27; choline, 24.5. Found: C, 19.20; H, 5.35; N, 2.83; P, 6.34; choline, 24.9. The air-dried cadmium chloride compound lost on drying *in vacuo* (0.1 mm.) over phosphorus pentoxide at 56°, 11.05% of its weight. Calcd. for the loss of three moles of water 10.92%.

Periodic Acid Titration.—The titration was carried out as described for the amorphous cadmium chloride compound of the synthetic diester: 0.0236 mM. of diester consumed 0.0229 mM. or 97% of periodate.

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Summary

1. A synthetic procedure is described by means of which the L- and D,L-α- glycerylphosphorylcholine have been prepared.

2. The synthetic L-α-glycerylphosphorylcholine was found to be identical with a product obtained from autolyzed beef pancreas by a slight modification of the purification procedure described by Schmidt, Hershman and Thannhauser.

3. The first successful application of monophenylphosphoryl dichloride as a phosphorylating agent is described. This reagent may prove useful in the synthesis of other phosphate-containing compounds of biological interest.

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(35) E. Fischer and E. Pfähler, *Ber.*, **53**, 1606 (1920); M. S. Newman and M. Renoll, THIS JOURNAL, **67**, 1621 (1945).