

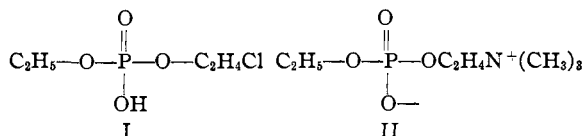
[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

The Synthesis of Choline and Amino-ethyl Esters of Ethyl Orthophosphate¹

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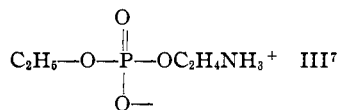
It is generally accepted that the phosphoric acid in the phospholipins, lecithin and cephalin, is connected to the glyceride and the nitrogen base through ester linkages, forming diesters of orthophosphoric acid.^{2,3} The naturally occurring phospholipins change very rapidly because of the ease of hydrolysis or oxidation.⁴ In order to study the properties of these substances due to the highly polar groups, phosphoric acid and nitrogen base, the simple diesters of orthophosphoric acid containing ethyl alcohol and either choline or amino-ethanol were synthesized.

The diesters of orthophosphoric acid were prepared by the method introduced by Langheld.⁵ The ethyl ester of metaphosphoric acid was treated with an equivalent of ethylene chlorohydrin forming the ethyl chloroethyl orthophosphate (I), which was isolated as the barium salt. When



this substance (I) was treated with an excess of trimethylamine in an alcoholic solution, the diester, ethyl choline orthophosphate, was formed (II) which may be isolated and purified by means of its flavianate. Langheld prepared the chloroplatinate of this substance and Abderhalden, Paffrath and Sichel⁶ prepared the hydrobromide. However, both of these authors state that their substance was impure.

Ethyl chloroethyl orthophosphate (I) reacted



(1) The results of this investigation were presented at the Chapel Hill, N. C., meeting of the American Chemical Society, April 11–16, 1937.

(2) Maclean and Maclean, "Lecithin and Allied Substances," Longmans, Green & Co., London, 1927.

(3) Levene, *Physiol. Rev.*, **1**, 327 (1921).

(4) Chain and Kemp, *Biochem. J.*, **28**, 2044 (1934).

(5) Langheld, *Ber.*, **44**, 2076 (1911).

(6) Abderhalden, Paffrath, and Sichel, *Arch. Ges. Physiol.* (Pflügers), **207**, 241 (1925).

(7) Evidence from dielectric constants of the natural products [Kuhn, Hausser, and Brydowna, *Ber.*, **68**, 2386 (1935)] and from dissociation constants, and solubilities of this synthetic compound in salt solutions, to be reported later from this Laboratory, indicate that this substance exists in this dipolar form.

with an excess of aqueous ammonia to form the diester, ethyl amino-ethyl orthophosphate (III). The pure crystalline substance was isolated and its monoflavianate prepared.

Plimmer and Burch⁸ found that Langheld's method of preparing esters of phosphoric acid gave a mixture of products. The principal impurities obtained by Langheld's method as used in this paper were derivatives of pyrophosphoric acid. These substances were separated by crystallizing the barium salt from aqueous solutions of alcohol, the pyrophosphoric acid derivatives being less soluble in aqueous alcohol than the orthophosphoric acid derivatives.

Experimental

Metaphosphoric Acid Ethyl Ester.—The method of Langheld was used to prepare the ester. A mixture of 200 g. of phosphorus pentoxide and an excess of dry diethyl ether was refluxed for ninety hours. The oily bottom layer dissolved in 400 cc. of chloroform was precipitated with dry ether. The precipitation by ether was repeated twice and yielded a sirupy product, largely ethyl metaphosphate contaminated with a small amount of diethyl pyrophosphate.

Ethyl Chloroethyl Orthophosphate (I).—To 100 g. of ethyl metaphosphate (1 mol.) was added 74.5 g. of ethylene chlorohydrin (1 mol.). After standing for four days at room temperature the barium salts were formed by neutralizing to litmus with a suspension of barium hydroxide crystals in water. During the addition of barium hydroxide the temperature of the solution was kept below 40°. On making the solution to 50% alcohol by adding absolute alcohol, a crystalline precipitate amounting to 33 g. separated. This substance proved to be the barium salt of diethyl pyrophosphate. After evaporating the filtrate under reduced pressure to a sirup, absolute alcohol was added to make the concentration of alcohol to 95%. A voluminous crystalline precipitate of the barium salt of ethyl chloroethyl phosphate formed, weighing 127 g. when dry. This material was further purified by two crystallizations from alcohol–water mixtures.

Anal. Calcd. for $(\text{C}_4\text{H}_9\text{PClO}_4)_2\text{Ba}$: P, 12.10; Cl, 13.83; Ba, 26.81. Found: P, 12.13; Cl, 13.70; Ba, 27.21.

Flavianate of Ethyl Choline Orthophosphate Ester.—To 20 g. of the barium salt of ethyl chloroethyl orthophosphoric acid (II) dissolved in 200 cc. of water was added an equivalent of sulfuric acid. After removing barium sulfate, the solution was concentrated under reduced pressure to a sirup, all water being removed by adding absolute methyl alcohol and evaporating several times. The residue was transferred to a pressure bottle with the use of

(8) Plimmer and Burch, *J. Chem. Soc.*, 292 (1929).

50 cc. of methyl alcohol and 60 cc. of a 33% solution of trimethylamine in alcohol added. The mixture was heated under pressure at 100° for twenty-four hours. Then, the excess trimethylamine was removed by evaporating under reduced pressure. Attempts to crystallize the hydrochloride of the diester at this point from organic solvents were not successful. The residue was dissolved in 100 cc. of water and made alkaline to a pH 10 with a solution of barium hydroxide, causing trimethylamine held in salt combination to be liberated. After the removal of trimethylamine by evaporation under reduced pressure, the solution was made to 90% alcohol. A precipitate formed which was largely barium ethyl chloroethyl phosphate and barium chloride. The precipitate was removed and the filtrate freed from barium and chloride ions by means of sulfuric acid and silver oxide. The solution was evaporated under reduced pressure until all water was removed and the residue dissolved in 100 cc. of absolute ethyl alcohol. The phosphoric acid ester of choline was precipitated by adding a saturated alcoholic solution of mercuric chloride. After standing for several days in the ice chest, 13.5 g. of the semicrystalline mercuric chloride derivative was obtained. The substance could not be recrystallized without adding an excess of mercuric chloride. The recrystallized mercuric chloride compound melted at 180–185° and the nitrogen-phosphorus ratio was 1:1. However, the percentage composition varied widely depending upon the manner of crystallizing, making the mercuric chloride derivative unsuitable for characterizing the substance.

The mercuric chloride derivative was dissolved in water and the mercury removed with hydrogen sulfide and the chloride with silver oxide. An unsuccessful attempt was made to obtain the free substance by evaporating the solution under reduced pressure to dryness and crystallizing the residue from organic solvents. Finally, the mono-flavianate was prepared by a procedure devised by Outhouse.⁹ To the product dissolved in 25 cc. of water was added 50 cc. of ethyl alcohol and 12 g. of flavianic acid. After adding 500 cc. of butyl alcohol, the solution was filtered, and the filtrate evaporated on a water-bath until crystals formed. After standing for several days in the ice chest, the crystals were filtered, and washed with butyl alcohol and ether. After drying, a yield of 9 g. of the flavianate was obtained. The flavianate was recrystallized twice from aqueous solutions containing ethyl and butyl alcohols. The substance did not have a sharp melting point. It began softening at 130° and finally melted at 155°. Flavianic acid was determined in the flavianate by the method of Langley and Albrecht.¹⁰ After the removal of flavianic acid, a dilute aqueous solution of the free ester was alkaline to litmus, having a pH of about 8.

Anal. Calcd. for $C_{17}H_{24}N_3O_{12}PS$: C, 38.83; H, 4.60; N, 8.00; P, 5.90; flavianic acid, 59.7. Found: C, 38.66; H, 5.09; N, 8.09; P, 5.75; flavianic acid, 59.1.

(9) Outhouse, *Biochem. J.*, **30**, 197 (1936).

(10) Langley and Albrecht, *J. Biol. Chem.*, **108**, 729 (1935).

Ethyl Amino-ethyl Orthophosphate (III).—Twenty grams of the barium salt of ethyl chloroethyl orthophosphate (I) was dissolved in water and the barium removed with an equivalent of sulfuric acid. The filtrate was concentrated to 50 cc. under reduced pressure and transferred to a pressure bottle, the total volume of liquid being about 80 cc. The pressure bottle containing the material was cooled to 0° and saturated with dry ammonia gas and then heated under pressure at 100° for twenty-four hours. Excess ammonia was removed by evaporating the solution under reduced pressure and the combined ammonia by making alkaline with barium hydroxide to pH 10.0 and concentrating at 40°. The residue was taken up with water and barium removed with sulfuric acid. The filtrate was concentrated to a sirup and crystallized from alcohol and acetone mixtures. A yield of 3 g. of ethyl amino-ethyl phosphoric acid diester was obtained. It was recrystallized from methyl alcohol and acetone mixtures. It melted at 228–230° and was very soluble in water, soluble in methyl alcohol, less soluble in ethyl alcohol, but insoluble in acetone and ether. A dilute aqueous solution of this substance is slightly acidic, having a pH of 5. The substance may also be prepared by treating ethyl chloroethyl phosphate with a large excess of ammonia at room temperature.

Anal. Calcd. for $C_4H_{12}O_4NP$: C, 28.38; H, 7.15; N, 8.28; P, 18.34. Found: C, 28.45; H, 7.37; N, 8.15; P, 18.38.

Flavianate of Ethyl Amino-ethyl Orthophosphate.—A slight variation of the method of Outhouse was used. To 1 g. of crystalline ethyl amino-ethyl phosphoric acid dissolved in 5 cc. of water was added 10 cc. of ethyl alcohol followed by 4 g. of flavianic acid and 100 cc. of butyl alcohol. After evaporating to one-half the original volume, a further addition of 100 cc. of butyl alcohol was made. Evaporation was continued until crystals began to form, after which the solution was transferred to the ice chest for complete crystallization. The yield of flavianate was practically quantitative. The flavianate was recrystallized twice, but did not have a sharp melting point. It softened at 120° and melted completely at 160°.

Anal. Calcd. for $C_{14}H_{18}N_3O_{12}PS$: C, 34.77; H, 3.75; N, 8.70; P, 6.42; flavianic acid, 64.9. Found: C, 34.25; H, 3.93; N, 8.62; P, 6.40; flavianic acid, 64.1.

Summary

The diesters, ethyl amino-ethyl and ethyl choline orthophosphate, have been synthesized as simple prototypes of the naturally occurring phospholipins, lecithin and cephalin. These substances have been prepared by the action of ammonia and trimethylamine upon ethyl chloroethyl orthophosphate. The flavianates of both compounds were prepared and the free ethyl amino-ethyl phosphoric acid was also obtained.

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