PHOSPHORYLETHANOLAMINE[1]

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ABSTRACT

A synthetic procedure is described that gives in excellent yield pure phosphorylethanolamine. Its X-ray diffraction pattern and infrared spectrum are reported.

Phosphorylethanolamine (PE) occurs in biological materials both in the bound and in the free state. The isolation of PE from malignant tumors by Outhouse (17), working with E. J. King in the Banting and Best Department, supplied the first evidence for the existence of free PE in nature. Subsequently it was also found in the intestines of rats and rabbits (13), in calf embryo muscle (15), in brain (2, 3, 22), and in various tissues of cows (24). Phosphorylethanolamine has been obtained by synthesis in yields ranging from 20% to 67%: (*a*) by phosphorylation of ethanolamine phosphate (17) or ethanolamine (19) with phosphorus oxychloride, (*b*) by phosphorylation of ethanolamine with a mixture of phosphorus pentoxide and phosphoric acid (19), (*c*) by treatment of phosphorylchloroethanol with ammonia (19), and (*d*) by the interaction of orthophosphoric acid and ethylenimine (11). The widely differing melting points reported for PE (228° (19), 230° (17), 232°–233° (19), 240° (11), and 244° (18, 12)) indicate, however, that these procedures give mixtures of organic phosphates from which it is apparently difficult to obtain the pure substance. In view of the biological interest that phosphorylethanolamine possesses it seemed desirable to develop a procedure for its synthesis that would give in good yield pure phosphorylethanolamine. This has been accomplished by substituting N-carbobenzoxyethanolamine for ethanolamine, and using diphenylphosphoryl chloride instead of phosphorus oxychloride as phosphorylating agent, thus preventing the formation of any by-product. The resulting O-diphenylphosphoryl carbobenzoxyethanolamine is freed of its protective benzyl and phenyl groups by consecutive catalytic hydrogenolysis with palladium in ethanol, and platinum in acetic acid, respectively. This procedure gives a chromatographically pure, crystalline phosphorylethanolamine in an over-all yield of 83%. Its melting point (244°–245°) agrees well with that reported by Outhouse (18) and Clarke *et al.* (12) for phosphorylethanolamine.

Attempts to simplify our procedure by removing the phenyl and carbobenzoxy groups by hydrolysis in boiling barium hydroxide solution were not successful, although the same procedure when applied to diphenylphosphorylcholine chloride (4) gives in good yield the barium salt of phosphorylcholine chloride. The protective carbobenzoxy group can be removed selectively, however, by the method of Albertson and McKay (1), using gaseous hydrogen bromide. The O-diphenylphosphorylethanolamine hydrobromide is obtained in a yield of approximately 90%. Since this compound might prove useful as a

[1]*Manuscript received December 1, 1955.*
Contribution from the Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario.

substrate in studies with phosphatases, its preparation is described in the experimental part.

In order to facilitate both the study of the structure and the quantitative estimation of natural phosphatides by infrared spectroscopy (14, 16, 21), the infrared spectra of pure individual phosphatides have been reported from this laboratory as these substances became available by synthesis (5, 6, 7). Possessing analytically pure specimens of phosphorylethanolamine and L-α-glycerylphosphorylethanolamine (8), both of which are of interest as moieties of cephalins and plasmalogens, their infrared spectra were recorded; these are shown in Fig. 1.

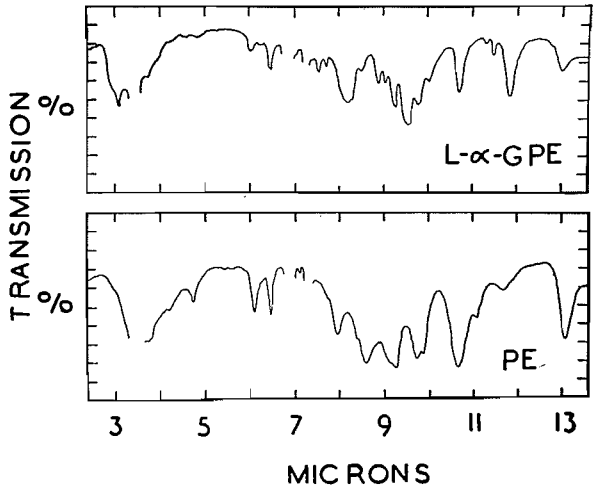
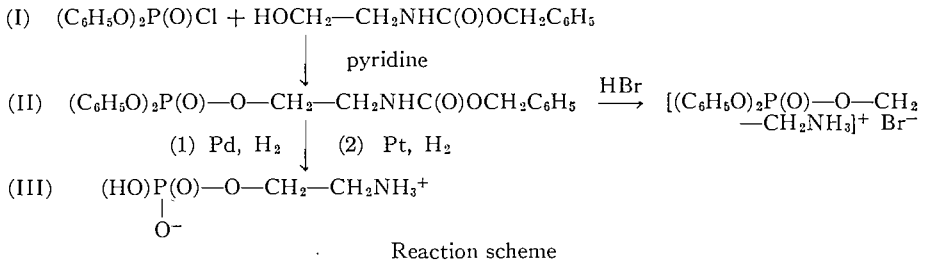


FIG. 1. Infrared spectra of phosphorylethanolamine (PE) and L-α-glycerylphosphorylethanolamine (GPE) in nujol mulls. Perkin-Elmer double-beam infrared spectrophotometer (model 21). Potassium bromide prism. Band positions and probable assignments: (PE) 2.90 μ (OH and NH stretching), 4.73, 6.08, 6.44 (NH bending), 7.10 (CH, OH bending), 7.94 (OH bending, P=O), 8.60 (C—O, C—N, C—C stretching), 9.25 (covalent phosphate), 9.72, 9.85, 10.64 (covalent phosphate), 11.08, 11.66, 13.08. (GPE) 3.10 μ (NH and OH stretching), 6.02, 6.48 (NH bending), 7.56 (CH, OH bending; primary and secondary alcohols), 7.74 (CH, OH bending; P=O), 8.18 (covalent phosphate), 8.55 (C—O, C—N, C—C stretching), 8.92, 9.08, and 9.30 (covalent phosphate), 9.58, 9.82, 10.06, 10.76 (covalent phosphate), 11.36, 11.52, 11.88, 13.06.



Reaction scheme

EXPERIMENTAL PART

O-(Diphenylphosphoryl)-carbobenzoxyethanolamine

To a solution of 9.75 gm. (5.0 mM.) of N-carbobenzoxyethanolamine (10,

20) in 40 ml. (50 mM.) of anhydrous pyridine* was added with swirling and under anhydrous conditions 14.8 gm. (5.5 mM.) of diphenylphosphoryl chloride (9) over a period of 10 min. To prevent the temperature from rising above 40°, the mixture was cooled occasionally with cold water. After it had been left for two hours at room temperature, the excess of diphenylphosphoryl chloride was destroyed by the addition of 2 ml. of water. One hour later the reaction product was isolated by precipitating it with 300 ml. of water. It was purified by washing its solution in 175 ml. of ether in succession with two 25 ml. portions of ice-cold 5 *N* sulphuric acid, and one 25 ml. portion each of water, a half-saturated solution of sodium bicarbonate, and water. After the solution had been dried over anhydrous sodium sulphate, it was concentrated under reduced pressure and the remaining solvent was removed by keeping the substance at 30°–35° in a vacuum of less than 1 mm. Hg for a period of five hours. The O-diphenylphosphoryl carbobenzoxyethanolamine, an almost colorless oil, weighed 20.4 gm. (95.4% of the theoretical amount based on carbobenzoxyethanolamine) $n_D^{23°}$ 1.5555. The substance is readily soluble at room temperature in ether, ethanol, methanol, or acetic acid but is insoluble in water. $C_{22}H_{22}O_6NP$ (427.2). Calculated: C, 61.81; H, 5.19; N, 3.29; P, 7.25. Found:† C, 61.79; H, 5.16; N, 3.30, 3.25; P, 7.12, 7.23.

Phosphorylethanolamine

A solution of 21.3 gm. (50 mM.) of O-diphenylphosphoryl carbobenzoxyethanolamine in 200 ml. of 99% ethanol together with 3.5 gm. of palladium catalyst (23) was shaken vigorously in an all-glass hydrogenation vessel of approximately 1 liter capacity in an atmosphere of pure hydrogen under a pressure of 40–50 cm. of water until the absorption of hydrogen had practically ceased. After replacing the hydrogen with nitrogen and adding 75 ml. of water to dissolve the reaction product, the catalyst was removed and the solution was brought to dryness under reduced pressure at a bath temperature of 25°–35°. To complete the removal of the protective groups, the solid residue was dissolved in 175 ml. of glacial acetic acid and the solution, after it was returned to the hydrogenation vessel and 3.5 gm. of platinic oxide‡ (Adams' catalyst) added, was shaken until there was no further uptake of hydrogen. After replacing the hydrogen with nitrogen and removing the catalyst, the acetic acid was distilled off *in vacuo* (bath temperature 30°–35°). The crude phosphorylethanolamine, containing traces of potassium, was purified by dissolving it in 350 ml. of distilled water and shaking the solution with 35 gm. of Amberlite IR–120 (H) for 90 min. The filtrate was brought to dryness *in vacuo* (bath temperature 30°–35°) and the phosphorylethanolamine was recrystallized from 35 ml. of water by gradually adding several volumes of 99% ethanol, and placing the mixture in an ice-box. The crystals were filtered off, washed with a small amount of a cold mixture of

*Pyridine of good commercial grade was refluxed over barium oxide and distilled with the exclusion of moisture.

†Analyses of two independent preparations.

‡The catalyst was prepared as described in "Organic Syntheses", Coll. Vol. 1, 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 463, with the exception that the sodium nitrate was replaced by an equimolecular amount of potassium nitrate.

ethanol and water (2:1), and dried *in vacuo* over phosphorus pentoxide. The phosphorylethanolamine weighed 6.0 gm. (87% of theory). Over-all yield 83%, m.p. 244°–245°.* $C_2H_8O_4NP$ (141.1). Calculated: C, 17.02; H, 5.71; N, 9.93; P, 22.0. Found: C, 17.10; H, 5.60; N, 9.88; P, 22.0.

X-ray Diffraction Pattern of Phosphorylethanolamine

The X-ray powder diffraction pattern was taken using Ni-filtered Cu K_α-radiation (λ 1.5418). The intensities of the diffraction rings were estimated visually on an arbitrary scale of 1 to 10 and are quoted in parentheses:
8.85 Å ($\frac{1}{2}$), 5.77 (7), 5.17 (4), 4.41 (10), 4.26 (10), 3.88 (8), 3.84 (8), 3.76 ($\frac{1}{2}$), 3.58 (5), 3.48 ($\frac{1}{2}$), 3.28 ($\frac{1}{2}$), 2.91 (1), 2.87 ($\frac{1}{2}$), 2.71 (3), 2.63 (2), 2.47 (3), 2.42 (1), 2.22 (3).

Diphenylphosphorylethanolamine Hydrobromide

Through a solution of 3.0 gm. of O-diphenylphosphoryl carbobenzoxyethanolamine in 20 ml. of glacial acetic acid was passed a stream of hydrogen bromide† gas for one-half hour. The solution was occasionally cooled. After the solution was left for one-half hour at room temperature, the solvent and excess of hydrobromide were removed under reduced pressure (8–10 mm. Hg) at a bath temperature of 35°–40°. To the solution of the residual oil in 5 ml. of 99% ethanol, ether was added until the solution was permanently turbid, and the mixture was placed in an ice-box overnight. The crystalline diphenylphosphorylethanolamine hydrobromide was collected with suction on a buchner funnel and was dried *in vacuo* over paraffin and calcium chloride. Yield 2.32 gm. (88% of theory), m.p. 115°–116.5° (with slight sintering at 114°). $C_{14}H_{17}O_4NPBr$ (374.2). Calculated: C, 44.93; H, 4.58; N, 3.74; P, 8.28. Found:‡ C, 44.96, 45.27; H, 4.75, 4.65; N, 3.79; P, 8.32.

This work was supported by a grant from the Lipotropic Research Fund, New York, to which the authors wish to express their sincere thanks. The authors express their thanks also to Professor E. W. Nuffield (Department of Geological Sciences, University of Toronto) for the X-ray diffraction measurements of phosphorylethanolamine, and to Dr. M. Look (Stanford University) for the infrared absorption spectra of phosphorylethanolamine and glycerylphosphorylethanolamine.

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**All melting points were determined in capillary tubes using an electrically heated bath of n-butyl phthalate and short-stem thermometers with a range of 50°.*

†The gaseous hydrogen bromide was prepared by the action of bromine on tetrahydronaphthalene.

‡Analyses of two independent preparations.

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