[CONTRIBUTION FROM THE DEPARTMENT OF NUTRITION, HARVARD SCHOOL OF PUBLIC HEALTH, AND THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Compounds Related to α-Glycerophosphoric Acid, Phosphorylcholine and Phosphorylethanolamine[1]

BY ARTHUR F. ROSENTHAL AND ROBERT P. GEYER

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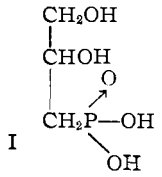
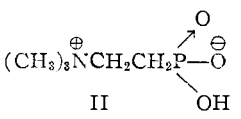
The synthesis of the phosphonate analogs of phosphorylcholine and of α-glycerophosphate is described. O-Phosphoryl-2-amino-2-methyl-1-propanol has been prepared by the polyphosphoric acid method. Also included in this paper is a direct preparation of glycerol α-iodohydrin from glycerol α-chlorohydrin.

The role of α-glycerophosphoric acid, phosphorylcholine and phosphorylethanolamine in the biosynthesis of lecithins and cephalins has been recently established.[2] Three analogs have now been synthesized for use as potential inhibitors of the biosynthesis of the glycerophosphatides.

Because of the chemical, and presumably also metabolic, stability of the carbon–phosphorus bond, it seemed of interest to prepare the phosphonate analogs of glycerophosphoric acid and phosphorylcholine.[3] The former compound (I) has been prepared as the barium salt by Arbuzov and Lugovkin[4] from epiiodohydrin and also from isopropylidene–glycerol α-chlorohydrin and the corresponding benzaldehyde 1,2-acetal. We have used the more readily available allyl bromide as the starting material. The glycol function was introduced smoothly *via* performic acid and the desired 1,2-propanediol-3-phosphonic acid isolated readily as the dilithium salt after basic hydrolysis.



I



II

Phosphonyldeoxycholine, or 2-(trimethylammonium)-ethanephosphonic acid (II), was prepared from diethyl vinylphosphonate by addition of dimethylamine and the methiodide of the diethyl dimethylaminoethanephosphonate hydrolyzed by acid. Although an attempt to apply the same synthesis to a higher homolog, 2-(triethylammonium)-ethanephosphonic acid, gave a product, no satisfactory analysis could be made on it.

Wells[5] has described an analog of ethanolamine, 2-amino-2-methyl-1-propanol, which competitively inhibits choline oxidase *in vitro* and brings about kidney degeneration *in vivo*. The phosphate ester of this substance has now been prepared by the polyphosphoric acid method of Cherbuliez and Weniger,[6] with a hydrolytic step added.

Finally, we have prepared glycerol α-iodohydrin in a simple one-step process from glycerol α-chlorohydrin and sodium iodide. The use of acetone as a solvent for the reaction has never been very satisfactory because of its reaction with the product.[7] In preliminary experiments we have found the same to be true of solvent acetonitrile. Nitromethane, however, shows no tendency to react, and the higher reflux temperature obtainable with this solvent avoids the use of pressure apparatus[8] and gives pure glycerol α-iodohydrin in good yield.

Experimental[9]

Dilithium 2,3-Dihydroxypropane-1-phosphonate.—Diethyl 2-propanephosphonate, b.p. 58–60° (1 mm.), was prepared in 81% yield from allyl bromide and triethyl phosphite.[10] To 35.6 g. (0.200 mole) of the ester were added 120 ml. of 88% formic acid and 35 ml. of 30% hydrogen peroxide. The mixture was warmed to 40° for a few minutes until its temperature rose above 42°. It was removed from the warming bath and intermittently immersed in a bath at 25° to keep the temperature at 40–45°. This exothermic phase lasted approximately 2 hr. The reaction was completed by keeping the mixture at 40–45° overnight.

The liquid was evaporated to a clear, colorless sirup (bath temperature, 70–80°; water pump) which was then heated on a steam-bath for 30 minutes. Hot, saturated aqueous lithium hydroxide was added slowly until a few drops of 1% phenolphthalein became red. Twenty ml. more of saturated lithium hydroxide was added, and after a few minutes a white precipitate began to form. The mixture was next heated in a steam autoclave at 120° for 5 hr., at the end of which time it became quite solidified. Enough water to make a total of 250 ml. was added and the mixture heated to boiling and filtered hot. Forty ml. more of hot, saturated lithium hydroxide was added and the liquid returned to the autoclave at 120° for 7.5 hr.

The precipitate on the funnel was washed three times with 95% ethanol, then with ether and air-dried. A second crop was isolated in the same way from the autoclaved filtrate. The combined yield of analytically pure 2,3-dihydroxypropane-1-phosphonate was 19.0 g. (62.5%). The substance is a fluffy white, non-hygroscopic powder. It is readily soluble in water, but like the lithium, calcium and barium salts of the corresponding α-glycerophosphoric acid, it is less soluble in hot than in cold water.

Anal. Calcd. for $C_3H_7O_5PLi_2$: C, 21.46; H, 4.20; P, 18.44; P:glycol, 1:1. Found: C, 21.62; H, 4.42; P, 18.36; P:glycol, 1:1.01.

Phosphorus in most of the compounds herein described was determined by the excellent method of Ma and McKinley.[11] We were able to confirm the statement of these authors that nitric–sulfuric acid is completely satisfactory

(1) Supported in part by a grant-in-aid from the American Cancer Society, and Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health.

(2) E. P. Kennedy, *J. Biol. Chem.*, **201**, 399 (1953); A. Kornberg and W. E. Pricer, Jr., *Federation Proc.*, **11**, 242 (1952); E. P. Kennedy and S. B. Weiss, *J. Biol. Chem.*, **222**, 193 (1956).

(3) The phosphorylethanolamine analog, 2-amino-ethanephosphonic acid, is already a well known substance. See G. M. Kosolapoff, THIS JOURNAL, **69**, 2112 (1947).

(4) B. A. Arbuzov and B. P. Lugovkin, *Zhur. Obshchei Khim.*, **22**, 1193 (1952).

(5) I. C. Wells, *J. Biol. Chem.*, **207**, 575 (1954); **217**, 631 (1955).

(6) E. Cherbuliez and H. Weniger, *Helv. Chim. Acta*, **29**, 2006 (1946).

(7) J. W. E. Glattfeld and R. Klaas, THIS JOURNAL, **55**, 1114 (1933).

(8) E. Baer and H. O. L. Fischer, *ibid.*, **70**, 609 (1948).

(9) Microanalyses, except most of the phosphate determinations, were performed by the Schwarzkopf Microanalytical Laboratory. All melting points were taken on a Fisher–Johns apparatus and are uncorrected.

(10) A. E. Arbuzov and A. I. Razumov, *Izvest. Akad. Nauk S.S.S.R. Otdel. Khim. Nauk*, 714 (1951).

(11) T. S. Ma and J. D. McKinley, *Mikrochim. Acta*, **1–2**, 4 (1953).

for the conversion of organic phosphorus to inorganic phosphate, although the diluted digestion mixture had to be heated above 100° for 30 minutes to obtain consistent results.[12]

(Trimethylammonium)-ethane-2-phosphonic Acid (Phosphonyldeoxycholine) (II).—To 5.0 g. of diethyl vinylphosphonate was added 10 ml. of 25% aqueous dimethylamine[13]; the clear homogeneous mixture became slightly warm. After standing for 24 hr. the mixture was evaporated *in vacuo*, then taken up in isopropyl alcohol (25 ml.) and re-evaporated. This was repeated once more.

The residual oil was dissolved in hexane (25 ml.) and dried over anhydrous magnesium sulfate for 12 hr. and filtered; the drying agent was washed with hexane (50 ml.). Five ml. of methyl iodide was added to the combined filtrate and washings. After standing for 24 hr., the precipitate was filtered, washed with hexane and dried *in vacuo;* yield 10.05 g. (94%). The substance after recrystallization from boiling isopropyl alcohol–ethyl acetate had a m.p. of 143–147° dec.

Three grams (0.0086 mole) of the diethyl dimethylaminoethanephosphonate methiodide was dissolved in 15 ml. of 48% hydrobromic acid and the mixture heated under reflux for 4 hr. As much hydrobromic acid as possible was removed *in vacuo* at steam-bath temperature. The residual reddish solid was taken up in water (25 ml.) and filtered. Silver oxide was added in small portions to the filtrate with vigorous stirring until the *p*H was 4.0–4.5. At this point a drop of the colorless solution gave no precipitate with either silver nitrate solution or dilute hydrochloric acid. The mixture was centrifuged until the supernatant was completely clear. It was evaporated until a white semi-solid remained. This was kept overnight in a high vacuum over phosphorus pentoxide.

The white crystalline solid was dissolved in 35 ml. of absolute methanol and centrifuged from any insoluble matter. Five volumes of anhydrous ether were added to the supernatant and the precipitate filtered. The precipitation was repeated and the product dried *in vacuo*. The powder, 1.6 g. (92% for the dihydrate), m.p. 227.5–228.5°, was recrystallized twice from boiling 95% ethanol–isopropyl alcohol, to give short colorless needles of the same melting point.

For analysis a few milligrams were dried at 50° in a high vacuum over phosphorus pentoxide.

Anal. Calcd. for $C_5H_{14}NPO_3 \cdot H_2O$: C, 32.43; H, 8.70; N, 7.56. Found: C, 32.38; H, 8.65; N, 7.64.

The conditions under which the dihydrate, monohydrate and the anhydrous compound are formed were somewhat difficult to reproduce, as they appear to depend in part on the amount of substance being dried, the vacuum obtainable, and the state of subdivision of the crystals. In one preparation the powder obtained by one ether precipitation appeared to be the monohydrate (P, 16.7%; calcd. 16.72%). After reprecipitation, or crystallization from 95% ethanol–isopropyl alcohol, the dihydrate was commonly formed (P, 15.48%; calcd. 15.24%). A few milligrams sent for analysis had, after drying at 80° *in vacuo* over phosphorus pentoxide, a phosphorus content of 18.22% and was evidently anhydrous (calcd. P, 18.53%).

Attempted Synthesis of 2-(Triethylammonium)-ethanephosphonic Acid.—The ethiodide of diethyl 2-diethylaminoethanephosphonate,[13] m.p. 128.5–129.5°, was prepared from the tertiary amine and ethyl iodide in trichloroethylene solution. After hydrolysis with 48% hydrobromic acid or constant-boiling hydrochloric acid and attempted isolation as in the above procedure, an oil remained which could not be crystallized. On adding hydriodic acid and evaporating to dryness, a red solid remained that was dissolved in water and decolorized with powdered silver. The solution after centrifugation was evaporated again and then recrystallized from methanol–acetone to give glistening colorless crystals, m.p. 245–246°, that rapidly darkened on exposure to air. It was probably the free (2-(triethylammonium)-ethanephosphonic acid) iodide, but as it seemed to lose part of its potential hydrogen iodide content very readily, satisfactory analyses could not be obtained.

O-Phosphoryl-2-amino-2-methyl-1-propanol.—2-Amino-2-methyl-1-propanol, Eastman practical grade, was redistilled. Five g. of the fraction of b.p. 75.5–76° (14 mm.) and 15.0 g. of polyphosphoric acid[14] were mixed and heated at 125° for 30 minutes with hand stirring. Seventy-five ml. of water was added; the mixture was stirred until homogeneous and heated for 2 hr. on a steam-bath, and a few drops of 1% phenolphthalein were added. A suspension of calcium hydroxide was added slowly with stirring until a faint pink color appeared. The calcium phosphate was filtered off with the aid of Hyflo-supercel, and saturated aqueous oxalic acid was added until the *p*H was 4.0. Calcium oxalate was removed by filtration, and the filtrate was evaporated to a thick sirup. Acetone (5 volumes) was added and the mixture allowed to stand for 2 hr. and filtered. The precipitate weighed 5.6 g. (59%).

Purification was effected by dissolving the ester in water (5 ml./g.) and adding the aqueous solution dropwise to a magnetically stirred mixture of 1:5 methanol–acetone (about 100 ml./g. of ester). The mixture was then heated to boiling for a few minutes in a hot water-bath, with swirling to prevent bumping and filtered. After three such reprecipitations the white powder had a m.p. of 238–239°.

Anal. Calcd. for $C_4H_{12}NO_4P$: C, 28.40; H, 7.15; N, 8.28; P, 18.31. Found: C, 28.66; H, 7.36; N, 8.09; P, 18.30.

Glycerol α-Iodohydrin.—Twenty-one ml. of glycerol α-chlorohydrin[15] (28 g., 0.25 mole) was dissolved in 250 ml. of nitromethane. Fifty g. of sodium iodide (0.33 mole) was suspended in the solution. The mixture was heated under reflux for 15 hr. and filtered; the precipitate was washed with minimal portions of nitromethane until white. The filtrate and washing were concentrated to a thick oil *in vacuo*. Separate washing of the precipitate repeatedly with acetone left 14.4 g. (98%) of sodium chloride.

Excess sodium iodide was removed by precipitation 4 times with ethyl acetate and ether, the solution decolorized with saturated aqueous sodium thiosulfate, dried and the solvent removed. The product was crystallized from chloroform–pentane at −5° to give 37 g. (74%) of product, m.p. 46–47°. Two recrystallizations raised the m.p. to 48–49°, undepressed on mixing with authentic glycerol α-iodohydrin prepared by the method of Baer and Fischer.[8]

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(12) Boiling stones should be used in the digestion mixture to avoid spattering. We have used alundum 12-B, Hengar silica granules and glass beads. The former two give a fine white precipitate of unknown composition during the digestion, but this completely disappears during the subsequent hydrolytic step and causes no interference in the formation of the phospho-vanado-molybdate complex.

(13) Diethyl vinylphosphonate was the gift of Dr. G. A. Richardson of the Monsanto Chemical Company. Diethyl dimethylaminoethanephosphonate and diethyl diethylaminoethanephosphonate may also be prepared from diethyl 2-bromoethanephosphonate. See G. M. Kosolapoff, THIS JOURNAL, **70**, 1971 (1948); and A. N. Pudovik and G. M. Denisova, *Zhur. Obshchei Khim.*, **23**, 263 (1953).

(14) Polyphosphoric acid, 115% orthophosphoric equivalent, was kindly supplied by the Victor Chemical Works.

(15) Glycerol α-chlorohydrin free from alpha, 2-dichlorohydrin, b.p. 82.5–83.5 (0.5 mm.), was prepared in 66% yield by the hydration of epichlorohydrin with aqueous sulfuric acid. A similar material may be purchased from the Matheson, Coleman, and Bell Company.