[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

Serine and Ethanolamine Diesters of Orthophosphoric Acid

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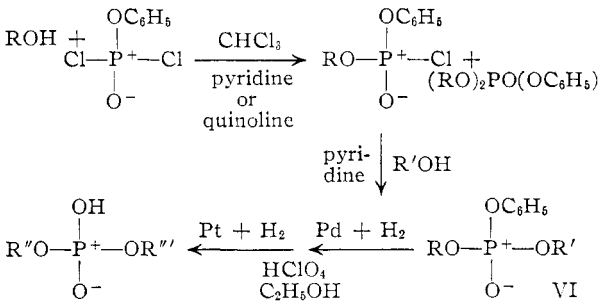
RECEIVED NOVEMBER 25, 1955

(2-Aminoethyl)-(L-2′-amino-2′-carboxyethyl)-phosphoric acid (I), the corresponding DL-serine derivative II, bis-(DL-2-amino-2-carboxyethyl)-phosphoric acid (III), bis-(2-aminoethyl)-phosphoric acid (IV) and the L-serine derivative corresponding to III (V) were prepared by techniques which lead to unequivocal structures for the products. Compound I, which was isolated as a formamide solvate, was shown to be identical in its paper chromatographic behavior with the phosphorus-containing compound recently isolated from turtle muscle by Roberts and Lowe. Furthermore compound I was hydrolyzed by snake venom in the same manner as the naturally-occurring compound.

Roberts and Lowe[1] recently described the isolation from turtle muscle of a new phosphorus-containing compound. This compound (I) was found by these authors to be a diester of orthophosphoric acid in which one of the ester groups is derived from L-serine and the other from ethanolamine. Using a series of reactions which have been successfully applied by Baer and his co-workers[2] to the preparation of similarly constituted compounds, we have succeeded in synthesizing I as well as the DL-serine derivative (II), bis-(DL-2-amino-2-carboxyethyl)-phosphoric acid (III) and bis-(2-aminoethyl)-phosphoric acid (IV). In addition, bis-(L-2-amino-2-carboxyethyl)-phosphoric acid (V) and III were isolated as by-products in the preparation of I and II, respectively. The I and II prepared synthetically were found to be identical in their behavior on paper chromatography with a sample of the naturally-occurring material isolated by Roberts and Lowe.[1,3] It also was found that I was hydrolyzed by snake venom in the same manner as the naturally-occurring compound.[4]

In order that the syntheses of the compounds mentioned above be unequivocal, it was necessary to use reactants that were suitably protected by blocking groups which eventually could be removed readily by a mild procedure. As shown in the diagrammatic representation of the reaction scheme



R and R′: $C_6H_5CH_2OCONHCH_2CH_2$—, DL- or L-$C_6H_5CH_2OOCCH(NHCOOCH_2C_6H_5)CH_2$—

I: R″, L-$HOOCCH(NH_2)CH_2$—; R‴, $H_2NCH_2CH_2$—

II: R″, DL-$HOOCCH(NH_2)CH_2$—; R‴, $H_2NCH_2CH_2$—

III: R″ and R‴, DL-$HOOCCH(NH_2)CH_2$—

IV: R″ and R‴, $H_2NCH_2CH_2$—

V: R″ and R‴, L-$HOOCCH(NH_2)CH_2$—

the amino groups of both the ethanolamine and serine were blocked by means of carbobenzoxy groups, while the carboxyl group of the serine was protected by conversion to the benzyl ester. Phenyl phosphorodichloridate was used as the blocked phosphorylating agent.[2,5] All of the blocking groups were removed from the intermediate neutral phosphate esters (VI) by hydrogenolysis, first in the presence of a palladium catalyst and then a platinum catalyst. The products (I to V) were purified by cellulose column chromatography and were isolated in satisfactory over-all yields, with one exception.

The bis-ethanolamine ester IV was isolated in good crude yield (65% based on ethanolamine), but the final yield of the product was poor. This apparently was due to the ease with which IV underwent rearrangement and decomposition on attempting to purify it by recrystallization or cellulose column chromatography. It was demonstrated by paper chromatography that when IV was allowed to stand at room temperature in sulfuric acid solution a product of different R_f was formed than when it was allowed to stand in sodium hydroxide solution. The fact that IV contains two basic groups and only one acidic group per molecule, whereas the other compounds (I, II, III and V) do not contain more basic than acidic groups, may be related to the ease with which IV is converted to other substances. Nitrogen–oxygen migrations of diisopropylphosphoryl[6] or acyl groups[7] in 1,2-aminoalcohols have been observed by other investigators. It is probable that the instability of IV is due to this type of migration.

Both of the products isolated in the preparation of IV (see Experimental section) are believed to be pure ethanolamine diesters of orthophosphoric acid, even though they each give two spots on paper chromatography in one of the solvent systems used. The multiple spots are believed to be due to different species, such as IV itself and the corresponding positive ion formed by the addition of a proton to IV. The formation of multiple spots on paper chromatography of pure amino acids has been observed.[8,9]

(1) E. Roberts and I. P. Lowe, *J. Biol. Chem.*, **211**, 1 (1954).

(2) E. Baer and M. Kates, THIS JOURNAL, **70**, 1394 (1948); E. Baer and M. Kates, *Science*, **109**, 31 (1949); E. Baer and M. Kates, THIS JOURNAL, **72**, 942 (1950); E. Baer, J. Maurukas and M. Russell, *Science*, **113**, 12 (1951); E. Baer, J. Maurukas and M. Russell, THIS JOURNAL, **74**, 152 (1952); E. Baer and J. Maurukas, *ibid.*, **74**, 158 (1952); E. Baer and H. C. Stancer, *ibid.*, **75**, 4510 (1953).

(3) We wish to thank Dr. Eugene Roberts for this sample and for some of the paper chromatographic data.

(4) Further studies on the enzymatic behavior of both natural and synthetic I are being pursued by Dr. Eugene Roberts.

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

(6) R. E. Plapinger and T. Wagner-Jauregg, THIS JOURNAL, **75**, 5757 (1953).

(7) J. A. Moore, J. R. Dice, E. D. Nicolaides, R. D. Westland and E. L. Wittle, *ibid.*, **76**, 2884 (1954); see also bibliography in reference 6.

(8) R. J. Block, E. L. Durrum and G. Zweig, "Paper Chromatography and Paper Electrophoresis," Academic Press, Inc., New York, N. Y., 1955, p. 78.

(9) E. Lederer and M. Lederer, "Chromatography," Elsevier Publishing Corp., New York, N. Y., 1953, pp. 208–209.

It is worth noting that the unequivocal synthesis of III yielded a product containing one-half mole of acetic acid and one-half mole of water of solvation, whereas the III obtained as a by-product in the preparation of II contained one mole of water of solvation but no acetic acid. This difference may be due to the fact that the latter sample was eluted from the cellulose powder column with water and the product powdered under acetone, whereas the former sample of III was eluted from the column with acetone–acetic acid–water and was not powdered under acetone. On the other hand, the L-isomer V which was isolated as a by-product in the preparation of I contained no solvate molecules.

One other interesting solvate was encountered during this work. Although II crystallized without solvent of crystallization, the L-isomer I contained one-half mole of water and one-half mole of formamide of solvation.

Experimental

Unless stated otherwise, melting points are uncorrected. They were determined using capillary tubes in a heated metal block.

Evaporation of solvents was carried out at room temperature *in vacuo* using a rotating evaporator (Rinco Instrument Co., Greenville, Ill.).

Solid samples were dried *in vacuo* (<0.1 mm.) over phosphorus pentoxide, except as noted below.

Infrared absorption spectra were obtained on Nujol mulls by means of a model 21 Perkin–Elmer infrared spectrometer with sodium chloride optics.

The R_f values reported are for descending chromatograms run on Whatman No. 1 paper using acetone–acetic acid–water (4:4:2, v./v.) as the developing solvent, except as noted below. The materials were detected on the papers by the use of a ninhydrin spray.[10]

The columns for cellulose powder chromatography were prepared by pouring a slurry of Whatman Standard Grade cellulose powder in acetone–acetic acid–water (4:4:2, v./v.) into a column of suitable dimensions. Air pressure was used to pack down the adsorbent. Before adsorbing the sample on the column, the cellulose powder was washed with the above solvent mixture until the effluent became colorless (usually about 1.5–2 l. of solvent).

Chloroform for use as a reaction medium was purified by distillation from phosphorus pentoxide. Anhydrous pyridine was prepared by distilling from a small amount of phosphorus pentoxide material which first had been dried over potassium hydroxide pellets.

The platinum and palladium oxide catalysts were prepared by the usual procedure,[11] except that potassium nitrate was used in place of sodium nitrate.[12] The 50% palladium-on-charcoal catalyst was prepared by the method of Linstead and Thomas.[13]

N-Carbobenzoxyethanolamine.[14]—The preparation of this compound in yields of 61–72% has been described previously.[15,16] It was obtained in 91% yield by keeping the *p*H of the reaction mixture between 9 and 10 during the addition of the benzyl chloroformate to the ethanolamine. The *p*H was kept between the desired limits by the addition, as needed, of 1 *N* sodium hydroxide to the well-stirred reaction mixture; λ_{max} 3.00, 4.45, 5.90, 6.45–6.50 (doublet), 7.85, 8.27, 8.70, 9.00, 9.48, 9.70, 10.10, 10.27, 10.4, 11.0, 11.9, 12.8, 13.4 and 14.4 μ.

N-Carbobenzoxy-DL-serine.—This compound was prepared by the method of Moore, *et al.*,[7] m.p. 123–125° cor. (86% crude yield). Melting points previously reported for this compound are 125°[17] and 124–125°[18]; λ_{max} 5.68, 5.90, 6.62, 7.48, 9.50, 13.3 and 14.4 μ.

N-Carbobenzoxy-L-serine.—The procedure of Moore, *et al.*,[7] was used for the preparation of this isomer from L-serine ($[\alpha]$ +14.3° in 1 *N* HCl, *c* 9.3) in a crude yield of 80%. After recrystallization from ethyl acetate, its physical constants (m.p. 117–118°, cor., $[\alpha]^{32}D$ +5.4° in glacial CH_3COOH, *c* 6) were in good agreement with previously reported values.[7,18,19]

N-Carbobenzoxy-DL-serine Benzyl Ester.—The preparation of this ester has been described by Baer and Maurukas. Prior to publication of this description, a procedure was developed which differs somewhat from the method used by these investigators. The silver salt of N-carbobenzoxy-DL-serine was precipitated from aqueous solution; m.p. 176.5–176.8°. *Anal.* Calcd. for $C_{11}H_{12}AgNO_5$: N, 4.05. Found: N (Kjeldahl), 4.21. A mixture of the dry silver salt, a moderate excess of benzyl chloride, and anhydrous acetonitrile (in which the silver salt is somewhat soluble) was refluxed for 18 hr. The desired benzyl ester (m.p. 74.0–74.4°) was recovered from this reaction mixture in 68% yield. Concentration of the mother liquors yielded another 1.9 g. of material, m.p. 72.0–73.0°. A melting point of 72.5–73.5° has been reported for this compound[18]; λ_{max} 3.05, 5.74, 5.94, 6.52, 7.40, 7.45, 7.96, 8.20–8.30 (broad), 9.26, 9.80, 12.2, 13.3 and 14.4 μ.

Anal. Calcd. for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.87; N, 4.25. Found: C, 65.46; H, 5.62; N, 4.22.

N-Carbobenzoxy-L-serine Benzyl Ester.—This compound was prepared by the procedure described above for the racemic compound in over-all yields of 48% (based on L-serine). The intermediate silver salt was isolated, m.p. 165.5–166.2°.

Anal. Calcd. for $C_{11}H_{12}AgNO_5$: N, 4.05. Found: N (Kjeldahl), 4.27.

The benzyl ester melted at 84.0–84.4° (previously reported[18] m.p. 84–85°), $[\alpha]^{32}D$ −8.7° (abs. C_2H_5OH, *c* 5), $[\alpha]^{26}D$ +5.4° (abs. $CHCl_3$, *c* 4). The latter rotation is in agreement with the value reported by Baer and Maurukas.[18]

Anal. Calcd. for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.87; N, 4.25. Found: C, 65.67; H, 5.62; N (Kjeldahl), 4.09.

(2-Aminoethyl)-(DL-2'-amino-2'-carboxyethyl)-phosphoric Acid (II).—Phenyl phosphorodichloridate (5.49 g., 0.025 mole) dissolved in 50 ml. of chloroform was cooled in an ice-bath. To this a solution of 8.24 g. (0.025 mole) of N-carbobenzoxy-DL-serine benzyl ester and 3.5 ml. (0.030 mole) of quinoline, in 25 ml. of chloroform was added, with stirring, over a period of 1 hr. After removing the ice-bath and stirring for another hour, the flask was immersed in a water-bath (20°) and 10 ml. (0.125 mole) of dry pyridine was rapidly added to the reaction mixture. This was followed by the addition over the course of an hour of a solution of 4.88 g. (0.025 mole) of N-carbobenzoxyethanolamine in 25 ml. of chloroform. The mixture was stirred overnight and then 100 ml. of ice-cold 6 *N* sulfuric acid was added. The chloroform layer was then washed successively with two more 100-ml. portions of ice-cold 6 *N* sulfuric acid, two 100-ml. portions of water, 0.5 *N* sodium bicarbonate solution, and once again with 100 ml. of water. After drying the resultant chloroform solution over anhydrous magnesium sulfate, evaporation of the solvent left an oily residue of a mixture of neutral phosphate esters (15.8 g., 95%) which could not be crystallized.

The hydrogenolysis of the neutral phosphate ester mixture was carried out at atmospheric pressure. A bulb containing Ascarite, to absorb carbon dioxide formed in the hydrogenolysis, was attached to the hydrogenation apparatus. The first stage of the hydrogenolysis, which involved the removal of the carbobenzoxy and benzyl groups, was carried out on a solution of 15.32 g. of the phosphate esters in 100 ml. of absolute ethanol plus 5 ml. of 10 *N* perchloric acid. Three grams of 50% palladium-on-charcoal was used as the catalyst. The uptake of hydrogen, which was complete in 6.5 hr., was 0.059 mole (86% of theory). The palladium catalyst was removed by filtration and washed successively with 10 ml. of water, 10 ml. of absolute ethanol and two 10-ml. portions of water. The filtrate and washings were re-

(10) See reference 8, p. 88.

(11) R. Adams, V. Voorhees and R. L. Shriner, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 463–470.

(12) A. H. Cook and R. P. Linstead, *J. Chem. Soc.*, 946 (1934).

(13) R. P. Linstead and S. L. S. Thomas, *ibid.*, 1127 (1940).

(14) We wish to thank Mr. Richard Wendt for assistance with the preparation of this compound.

(15) E. Chargaff, *J. Biol. Chem.*, **118**, 417 (1937).

(16) W. G. Rose, THIS JOURNAL, **69**, 1384 (1947).

(17) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(18) E. Baer and J. Maurukas, *J. Biol. Chem.*, **212**, 25 (1955).

(19) J. S. Fruton, *J. Biol. Chem.*, **146**, 463 (1942).

turned to the hydrogenation apparatus, 1.5 g. of platinum oxide was added, and hydrogenolysis was resumed. The uptake of hydrogen in this second stage amounted to 0.222 mole (75% of theory, if the hydrogenation of the toluene formed in the first stage is taken into account) and was complete in about 5 hr.

After the hydrogenolysis of the neutral esters was completed, 16.7 ml. of 3 *N* sodium hydroxide was added to the reaction mixture and the catalyst was removed by filtration. The filtrate was concentrated, extracted with ether, and then evaporated to dryness. The solid residue, which was shown by paper chromatography to contain several components giving a positive ninhydrin reaction, was dissolved in 25 ml. of acetone–acetic acid–water (4:4:2, v./v.) and was adsorbed on a cellulose powder column 96 cm. long by 4.6 cm. in diameter. Material was eluted from the column first with 5.4 l. of the above solvent (flow rate 40 ml. per hour) and then with 3.0 l. of water. The acetone–acetic acid–water effluent was collected in 10-ml. fractions, while the aqueous effluent was collected in 100-ml. fractions. Fractions 307–540, inc., of the acetone–acetic acid–water effluent were combined and evaporated to dryness. The residue was dissolved in water and the resulting murky yellow solution was filtered. A fine, white crystalline precipitate was obtained by the addition of ethanol to the aqueous filtrate. This was removed by filtration, washed with absolute ethanol, and dried (2.13 g.). Recrystallization of this material from aqueous alcohol gave 1.98 g. of product (36% yield based on N-carbobenzoxy-DL-serine benzyl ester), m.p. 180–181° dec. Paper chromatography in four solvent systems showed that the product was homogeneous and identical in its behavior with naturally-occurring I. The solvent systems used and the R_f values found are: (1) acetone–acetic acid–water (4:4:2, v./v.), R_f 0.24; (2) acetone-acetic acid–water (4:2:2, v./v.), R_f 0.85[3]; (3) water-saturated phenol in the presence of acetic acid vapor, R_f 0.31[3]; and (4) *n*-butyl alcohol–acetic acid–water (8:2:2, v./v.), R_f 0.18[3]; λ_{max} 3.76, 4.28, 4.76, 6.08, 6.26, 6.54, 7.08, 7.44, 7.60, 7.70, 8.08, 8.20, 8.58, 8.78, 9.30, 9.66, 9.95, 10.06, 10.75, 11.26, 12.02, 12.62 and 13.26 μ.

Anal. Calcd. for $C_5H_{13}N_2O_6P$: C, 26.32; H, 5.74; N, 12.28; amino N, 12.28; P, 13.58. Found: C, 25.85; H, 5.51; N (Kjeldahl), 12.13; amino N (Van Slyke), 12.18; P, 13.45.

Fractions 5–31, inc., of the aqueous effluent from the cellulose column were combined and evaporated to dryness. Since attempts to crystallize the glassy solid which remained failed, it was redissolved in water, the aqueous solution was filtered, and then evaporated once again to dryness. The solid residue was powdered under acetone and the resulting powder was dried *in vacuo* over activated alumina. The product was a white powder weighing 0.84 g., m.p. 120–121° dec. On paper chromatography it gave only one spot, R_f 0.13. This is identical with the R_f value obtained for the III whose preparation is described below.

Anal. Calcd. for $C_5H_{13}N_2O_6P \cdot H_2O$: C, 24.83; H, 5.21; N, 9.64; P, 10.67. Found: C, 24.62; H, 5.17; N (Kjeldahl), 9.71; P, 10.67.

(2-Aminoethyl)-(L-2′-amino-2′-carboxyethyl)-phosphoric Acid (I).—Using N-carbobenzoxy-L-serine benzyl ester (8.24 g., 0.025 mole), instead of the DL-compound, the mixture of neutral phosphate esters was prepared as described under the preparation of II. This mixture, which was obtained in 92% yield, was a yellow oil, $n^{32}D$ 1.5452, $[\alpha]^{32}D$ −4.0° (abs. C_2H_5OH, *c* 5.5).

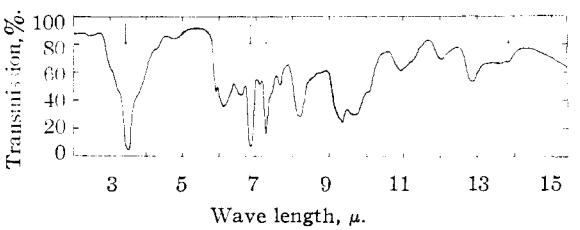


Fig. 1.—Infrared absorption spectrum of (2-aminoethyl)-(L-2′-amino-2′-carboxyethyl)-phosphoric acid in a Nujol mull. The arrows indicate the principal absorption bands of the Nujol.

In order to remove catalyst poisons from the mixture of neutral phosphate esters which interfered with its hydrogenolysis, it was dissolved in chloroform and the solution was washed twice with water. The chloroform solution was then shaken with a mixture of acid-washed charcoal (Norit A) and anhydrous magnesium sulfate. After filtration, the chloroform was evaporated to recover the purified ester mixture. A sample of this material (10.05 g.) was subjected to hydrogenolysis as described above. In the first step of the hydrogenolysis, using 50% palladium-on-charcoal, 0.0398 mole of hydrogen (87% of theory) was consumed in 8 hr. The second step, using a platinum oxide catalyst, consumed 0.144 mole of hydrogen (68% of theory) in about 7 hr. The solution obtained after the hydrogenolysis was neutralized with aqueous sodium hydroxide, was concentrated to a sirup, and then was chromatographed on a cellulose powder column as described under the preparation of II. The fractions containing I were combined and evaporated to dryness. Since paper chromatography showed that several components were present in the product thus obtained (2.0 g.), it was rechromatographed on a cellulose powder column 100 cm. long and 4.6 cm. in diameter. The fractions containing the desired product were pooled and evaporated to dryness. The horny residue was dissolved in water and the resulting solution was filtered. The clear filtrate was once again evaporated and the solid which remained was powdered. It was dissolved in 25 ml. of pure, anhydrous formamide and 150 ml. of absolute ethanol was added to the solution. The flocculent white precipitate which formed was recovered by centrifugation and was washed with two 20-ml. portions of absolute ethanol and then dried *in vacuo*. To the dried material (1.5 g.), which consisted of a mixture of a white powder and a yellow gum, was added 25 ml. of formamide. The mixture was centrifuged and 100 ml. of absolute ethanol was added to the clear supernatant. The flocculent precipitate which formed was recovered by centrifugation and washed three times with 50-ml. portions of absolute ethanol. The product, which was dried to constant weight at room temperature and 10^{-5} mm. pressure, weighed 0.90 g. (21% over-all yield based on N-carbobenzoxy-L-serine benzyl ester), m.p. 139–141° dec., $[\alpha]^{23.5}D$ −15.0° (H_2O, *c* 2.2). The behavior on paper chromatography of the synthetic (R_f 0.22) and natural (R_f 0.21) products was identical and the same as that of II. Both samples gave single, ninhydrin-positive spots. The infrared absorption spectrum of the synthetic material is reproduced in Fig. 1.

Anal. Calcd. for $2C_5H_{13}N_2O_6P \cdot HCONH_2 \cdot H_2O$: C, 25.44; H, 6.02; N, 13.49; amino N, 10.79[20]; P, 11.93. Found: C, 25.22; H, 5.57; N (Kjeldahl), 13.25; amino N (Van Slyke), 10.90; P, 11.94.

It was possible to recover bis-(L-2-amino-2-carboxyethyl)-phosphoric acid (V) from the aqueous effluent from the cellulose powder column. The fractions containing V were combined and evaporated to dryness. A glassy residue remained (0.60 g.) which was shown by paper chromatography to contain two components. Rechromatography on a cellulose powder column (72 cm. long, 3 cm. in dia.) yielded a glassy product which weighed 0.41 g., m.p. 125° dec., $[\alpha]^{23.5}D$ −11.6° (H_2O, *c* 2.0), R_f 0.12. The R_f value is in good agreement with the corresponding value for III; λ_{max} 4.15, 4.40, 5.80, 6.20, 8.65, 9.75, 10.3, 11.25 and 13.0 μ. The spectrum was actually a continuum with the above weak absorption bands superimposed on it.

Anal. Calcd. for $C_6H_{13}N_2O_8P$: C, 26.48; H, 4.81; N, 10.29; amino N, 10.29; P, 11.38. Found: C, 26.48; H, 5.53; N (Kjeldahl), 10.29; amino N (Van Slyke), 10.42; P, 11.04.

Bis-(DL-2-amino-2-carboxyethyl)-phosphoric Acid (III).—A flask containing 16.47 g. (0.050 mole) of N-carbobenzoxy-DL-serine benzyl ester, 4.1 ml. (0.055 mole) of pyridine and 150 ml. of chloroform was cooled in an ice-bath and a solution of 5.27 g. (0.025 mole) of phenyl phosphorodichloridate in 25 ml. of chloroform was added, with stirring, over a period of 1 hr. The ice-bath then was removed and the reaction mixture was stirred at room temperature overnight.

(20) Formamide does not evolve appreciable amounts of nitrogen in the usual Van Slyke determination, which gives quantitative results with the amino acids and the compounds which are described in this paper. Ammonium formate, on the contrary, yields about 25% of the theoretical amount of nitrogen under these same conditions.

At the end of this time interval, the reaction mixture was washed successively with two 100-ml. portions of ice-cold 6 *N* sulfuric acid, 100 ml. of cold water, 100 ml. of 0.5 *N* sodium bicarbonate and twice with water. The resulting chloroform solution, after drying over anhydrous magnesium sulfate, was evaporated to constant weight. The product (19.9 g., 100% yield) was a viscous, yellow oil (n^{35}D 1.5509) which could not be crystallized.

Hydrogenolysis of the neutral phosphate ester mixture was carried out in two stages as described previously. The mixture (16.85 g.) was dissolved in 100 ml. of absolute alcohol plus 4.5 ml. of 10 *N* perchloric acid and 1.63 g. of palladium oxide catalyst was added. Hydrogen uptake (0.082 mole, 83% of theory) was complete in 2.5 hr. The resulting solution was filtered free of catalyst and the catalyst washed with 10 ml. of ethanol and 30 ml. of water. To the combined filtrate and washings were added 1.71 g. of platinum oxide catalyst and the mixture once again was subjected to hydrogenolysis. At this stage another 0.220 mole of hydrogen (63% of theory) was consumed in 4.5 hr. After adding 15 ml. of 3 *N* sodium hydroxide to the reaction mixture, the catalyst was removed by filtration and the filtrate was concentrated. The resulting aqueous solution, which was shown by paper chromatography to contain three ninhydrin-reactive substances, one of which was serine, was subjected to chromatography on sheets of Whatman No. 4 filter paper. The material which was recovered from the zone of smallest R_f was a white, glassy solid (2.06 g.). Since it was still contaminated with a substance of higher R_f, it was rechromatographed on a cellulose powder column (97 cm. long by 4.6 cm. dia.) using acetone–acetic acid–water (4:4:2, v./v.) as the eluent. The effluent was collected in 50-ml. fractions at the rate of approximately 1.3 ml. per min. The fractions containing III (102–231, inc.) were combined and evaporated to dryness. The residue was dissolved in water and the aqueous solution, after filtration, once more was evaporated to dryness. The resulting glassy residue was powdered and dried. The product weighed 1.83 g. (28% yield based on N-carbobenzoxy-DL-serine benzyl ester), m.p. 115° dec., R_f 0.13. A minor impurity (R_f 0.8) was found to be present by paper chromatography; λ_{max} 5.78, 6.15, 8.20, 8.65, 9.70, 10.2, 11.2 and 13.1 μ. The spectrum was actually a continuum with the above weak absorption bands superimposed on it.

Anal. Calcd. for $2C_6H_{13}N_2O_5P \cdot CH_3COOH \cdot H_2O$: C, 27.02; H, 5.18; N, 9.00; amino N, 9.00; P, 9.96. Found: C, 26.88; H, 4.75; N (Kjeldahl), 9.15; amino N (Van Slyke), 8.99; P, 10.21.

Bis-(2-Aminoethyl)-phosphoric Acid (IV).—A solution of 11.7 g. (0.060 mole) of N-carbobenzoxyethanolamine in 5.2 ml. (0.066 mole) of pyridine and 100 ml. of chloroform was cooled in an ice-bath and 6.3 g. (0.030 mole) of phenyl phosphorodichloridate in 25 ml. of chloroform was added dropwise, with stirring, over the course of 1.25 hr. After the addition of the phosphorodichloridate was completed, the reaction mixture was stirred at room temperature overnight. It then was washed successively with two 100-ml. portions of ice-cold 6 *N* sulfuric acid, 100 ml. of cold water, 100 ml. of 0.5 *N* sodium bicarbonate and two 100-ml. portions of water. The solvent was evaporated from the resulting chloroform solution after drying over anhydrous magnesium sulfate. The residue of neutral phosphate esters (13.6 g., 86% yield) was a yellow viscous oil, n^{33}D 1.5440.

The hydrogenolysis of this mixture of neutral phosphate esters was carried out as described previously, using 11.02 g. (0.021 mole) of ester dissolved in a mixture of 100 ml. of absolute ethanol and 4.5 ml. of 10 *N* perchloric acid. The first step of the hydrogenolysis, in which 2.2 g. of 50% palladium-on-charcoal was used as the catalyst, required *ca.* 20 hr. for the consumption of 0.034 mole (82%) of hydrogen. After removal of the palladium catalyst by filtration, the second stage of the hydrogenolysis was carried out using 1.12 g. of platinum oxide catalyst. This stage consumed 0.164 mole (76% of theory) of hydrogen in 5 hr. The platinum catalyst was removed by filtration after 15 ml. of 3 *N* sodium hydroxide was added to the mixture. A white solid separated on storing this neutralized solution overnight at −20°. The precipitate, which was removed by filtration after warming the mixture to room temperature, was air-dried (0.35 g.). Recrystallization from ethanol–water (5:1) yielded 0.28 g. of product, m.p. 233–235°. Paper chromatography with the usual acetone–acetic acid–water solvent system gave one spot, R_f 0.59. Using 95% ethanol–1 *M* ammonium acetate (75:30, v./v.), however, two spots were obtained with R_f values of 0.45 (very weak) and 0.68 (intense).

Anal. Calcd. for $C_4H_{12}N_2O_4P \cdot HClO_4$: C, 16.88; H, 4.97; P, 10.89. Found: C, 16.80; H, 4.91; P, 11.10.

The filtrate was shown by paper chromatography to contain three ninhydrin-reactive substances with R_f values of 0.76 (ethanolamine), 0.59 and 0.48. It was evaporated to dryness and the solid residue which remained was taken up in water. Addition to this aqueous solution of six volumes of ethanol brought about the formation of a crystalline precipitate. It was removed by filtration and air-dried after washing first with absolute alcohol and then ether. The product weighed 3.49 g., m.p. 221–223°. Attempts to further purify this sample by recrystallization or cellulose column chromatography, using acetone–acetic acid–water (4:4:2, v./v) as the eluent, led to extensive conversion to substances with lower R_f values (*ca.* 0.4) and ethanolamine. It was possible, however, to isolate 2.3 g. of crystalline solid from a series of fractions from the cellulose column which contained material of R_f *ca.* 0.4. This solid was dissolved in 15 ml. of warm water. After filtration to remove insoluble material, the aqueous solution was diluted with 60 ml. of ethanol and then 40 ml. of ether. Fine, hard crystals formed which were removed by filtration and dried. The sample weighed 0.9 g., m.p. 235–236.6°. This was recrystallized from 5 ml. of water by dilution first with 25 ml. of ethanol and then 15 ml. of ether. The fine, white crystals which formed were removed by filtration and washed with ether. After drying *in vacuo*, the product weighed 0.78 g., m.p. 234–235°. Paper chromatography of this material in the usual acetone–acetic acid–water solvent system gave two spots with R_f values of 0.43 (intense) and 0.60 (moderately intense). With 95% ethanol:1 *M* ammonium acetate as the developing solvent, only one spot was obtained (R_f 0.70); λ_{max} 4.75, 6.10, 6.48, 7.80, 8.35, 8.75, 9.30, 9.90, 10.2, 10.8 and 12.6 μ.

Anal. Calcd. for $3C_4H_{12}N_2O_4P \cdot HClO_4$: C, 22.07; H, 6.18; N, 12.87; amino N, 12.87; P, 14.24. Found: C, 21.79; H, 6.25; N (Kjeldahl), 12.71; amino N (Van Slyke), 12.72; P, 14.55. The presence of chlorine in the compound was demonstrated by means of a sodium fusion.

Acknowledgment.—This research was supported in part by the U. S. Atomic Energy Commission. The microanalytical work was performed by Mrs. Charlotte P. Thompson.

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